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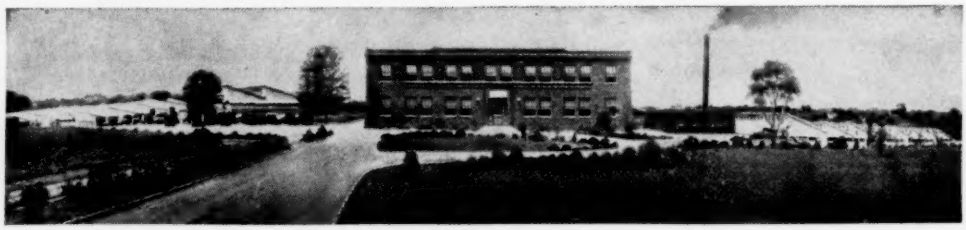

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Vol. XCV DECEMBER, 1939 No. 753

Improved Methods in the Diagnosis of Rabies*

By JOSEPH C. WILLETT, D.V.M., and S. EDWARD SULKIN, Ph.D.

St. Louis, Mo.

WHEN guinea pigs are injected with material for the diagnosis of rabies, irregularities of the incubation period are frequently encountered, and the animals often survive the injections. The need for a more desirable test animal is obvious.

COMPARATIVE SUSCEPTIBILITY OF GUINEA PIGS AND MICE TO RABIES VIRUS

Despite reports that mice have proved unsatisfactory as test animals, recent studies of Webster and his associates,[1, 2, 3] indicate that certain inbred strains of mice are highly desirable for diagnostic purposes. A diagnostic test by mouse inoculation was developed by Webster and Dawson[4] as an aid to the microscopic method. These investigators used a strain of albino mice distinguished because of their greater susceptibility to neurotropic viruses. The technic described by these investigators was only slightly modified in the present study. An approximate 10 per cent suspension of brain tissue from the central portion of the hippocampus major was made. Salt solution was used as the diluent and larger clumps were removed by permitting them to settle in the mortar rather than by centrifuging them. Throughout this study guinea pigs received 0.1 cc. of the 10 per cent brain suspension intracerebrally while mice received 0.03 cc. When intramuscular injections were made, 0.25 cc. of the suspension was introduced.

Impression smears were made of Ammon's horn and a portion of the cerebellum. A thorough search was made for Negri bodies. Throughout this study the modification of Van Giesen's stain for Negri bodies, described by Nagle and Pfau,[5] was used.

For several years a procedure was followed in our laboratory whereby all animal brains reported negative or doubtful on the basis of microscopic examination were injected intracerebrally into each of two guinea pigs in order to confirm the

*From the laboratories of the St. Louis health division. Presented before the Section on Sanitary Science and Food Hygiene at the 76th annual meeting of the A. V. M. A., Memphis, Tenn., August 28 to September 1, 1939.

diagnosis. During the past five years, however, animal inoculations have been made only when requested by the epidemiologist.

An analysis of our records on 1,998 animal brains submitted to the laboratory for examination from 1921 to 1938 revealed the following facts. Of the total number of brains received, 997 specimens were reported positive, 945 negative and 56 doubtful. Of the 945 specimens reported negative on the basis of microscopic examination, 29, or 3.07 per cent, proved positive on guinea pig inoculation, while twelve, or 21.4 per cent, of the 56 doubtful specimens proved positive on animal inoculation. The average number of days from the time of injection until the time of death of the first guinea pig of each set was 19.7. Incubation periods varied from eight to 59 days.

In order to determine the relative susceptibility of guinea pigs and white mice to rabies virus, animals were injected with brain emulsion from 27 dog brains showing Negri bodies on microscopic examination. The brain emulsion from each was injected intracerebrally into two young (200 Gm. [6.7 oz.]) and one old (350-450 Gm. [12-15 oz.]) guinea pigs, and into each of four young (4-6 weeks of age) white mice. The animals were observed for a period of nine weeks. None of the animals was examined for Negri bodies if death took place within three days after the injection. These deaths were considered to be due to trauma. In this group of 81 guinea pigs only one (1.23 per cent) died from trauma, while of 104 mice mortality due to mechanical injury was 2.87 per cent.

The results of these comparative experiments, which have been published recently,[6] suggest that the mouse is more susceptible than the guinea pig to rabies virus. Only 9.3 per cent of the 104 mice failed to develop rabies, while 22.2 per cent of the 54 young guinea pigs and 44.4 per cent of the 27 old guinea pigs survived. The average number of days from the time of injection until the time of death of mice showing Negri bodies was 13.6 days; the number of days for guinea pigs was 18.4. Furthermore, the first mouse of the series died in an average of eleven days; the first guinea pig died in an average of 14.5 days. Of the 27 specimens reported positive for Negri bodies, two proved negative by the mouse method, while six proved negative by the guinea pig-inoculation method.

Comparative Susceptibility of St. Louis Stock Mice and Swiss Mice

Since St. Louis mice* were used in these experiments, due to the limited number of Swiss mice available at the time, an effort was made to determine the relative susceptibility of the St. Louis stock mice and the inbred strain of Swiss mice. Ten microscopically positive dog brains were used in these experiments. The same supernatant fluid from each specimen was injected intracerebrally and intramuscularly into mice of the two groups. One mouse from each group was sacrificed on the sixth, seventh and ninth days, respectively. Impression smears of Ammon's horn and the cerebellum were examined for Negri bodies. The results of this experiment showed only a slightly greater susceptibility of the Swiss mice to the rabies virus. The St. Louis stock mice died in an average of twelve days, while the Swiss mice died in an average of 11.2 days. Furthermore, the first animal of the St. Louis group died or was found positive in 9.2 days while the first Swiss mouse died or was found positive in 8.9 days.

Demonstration of Rabies Virus in Grossly Decomposed Animal Brains

A suitable and rapid animal-inoculation method is desirable in order to obviate difficulties encountered in the examination of putrified brain tissue submitted to the laboratory for rabies diagnosis.

In order to attempt demonstration of virus in decomposed brains it became necessary to remove the bacterial contaminants from the putrid material, since intracerebral injection of such material into mice invariably produced death from intercurrent infection or from brain abscess. In

*The animals referred to as St. Louis stock mice consist of three different strains obtained from St. Louis dealers.

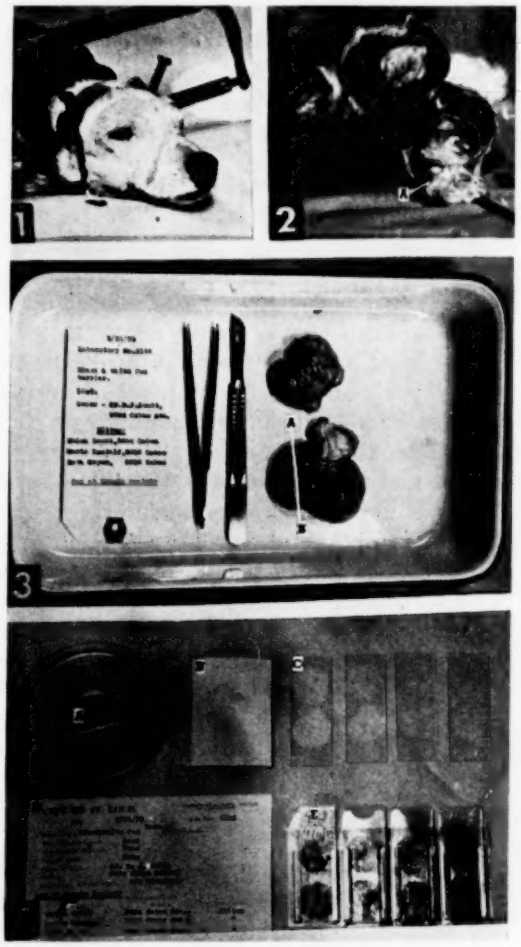
the preliminary experiments several bactericidal agents, including merthiolate, glycerine, phenol and ether, were tried in an effort to find one which would sterilize the heavily infected inoculum without appreciably affecting the virus. Ether used according to the method suggested by Kramer and his associates,[7] Paul and Trask[8] and others proved to be the most desirable agent because its extreme volatility permitted prompt removal from the contaminated material after its bactericidal action had taken effect.

In experiments reported previously[9] it was found that ether in final concentration of 10 per cent exerted a definite bactericidal effect upon contaminated dog brains after exposure for two hours at 4° C. Furthermore, this concentration had no effect upon the virulence of the strains of street virus tested.

The following method is recommended for treating contaminated brains before intracerebral injection into animals. The central portion of the hippocampus major (cornu ammonis) is dissected out and a small piece removed for microscopic examination and for glycerination. The remaining portion of the horn is emulsified in 10 cc. of nutrient broth and allowed to stand for one hour at room temperature. The supernatant liquid (after larger clumps have settled) is pipetted into a stoppered 15-cc. (½-oz.) centrifuge tube and 10 per cent ether (about 1 cc.) is added. The tube is then shaken thoroughly and stored in the refrigerator for two hours. After centrifugation at low speed the supernatant liquid below the ether layer is removed for animal inoculation.

A Simple Method for the Removal of Animal Brains

The method now used extensively in many state and municipal health department laboratories for the removal of animal brains for the diagnosis of rabies involves the use of a hammer, chisel, bone forceps and meat cleaver. The manipulations with such instruments may result in spattering of infected bone or brain tissue. The technic frequently used consists of making a median longitudinal incision through the skin fascia and muscles of the cranium, and extending the incision to the level of the eyes and laterally from the occipital protuberance, permitting soft tissue to be reflected on both sides. The top of the skull is then removed after chiseling or sawing through the bone, making a circular opening. Fre-



Figs. 1-4. A simplified technic for the removal of dog brain for the diagnosis of rabies. 1) The head is stationed firmly with nails and the saw passed sagittally through the parietal bones. 2) This shows the inner surface of the excised portion, the brain (A) having been removed from the cranial cavity. 3) The instruments shown in this photograph are used to expose the hippocampus major (A-B). At the left is the laboratory record of the case, which gives the breed of the dog, the name and address of its owner and the names and addresses of those whom it has bitten. 4) A, Petri dish containing Ammon's horn; B, paper-covered glass slide (2"x3") for making impressions; C, impression films; D, permanent record card; and E, stained films.

quently, removal of the top of the skull is facilitated by sawing longitudinally through the parietal bones and transversely through the skull just above the eyes. The opening is sometimes made larger by breaking away the sides of the skull with bone forceps. This method, although satisfactory, is time-consuming and cumbersome.

The simplified method here presented (see composite illustration) requires the use of the following instruments: 1) A heavy board (2″x12″x16″) with two 6-inch nails located 2 inches from one end and about 4 inches apart; 2) one scalpel (Bard-Parker No. 4 handle, No. 20 blade); 3) one pair of rubber gloves; 4) one tablespoon; and 5) one metal adjustable hack saw (8-inch blade).

The animal is placed on the operating table with its head between the nails on the paper-covered heavy board. The surface of the head is then thoroughly moistened with 4 per cent cresol. With a heavy scalpel a sagittal incision is made midway between the eyes and ears, cutting through the skin, fascia and muscles of the cranium. The brain may be exposed by sawing sagittally through the parietal bones in the region of the fronto-parietal suture (fig. 1). The nerves on the under side of the cerebrum, tentorium, and spinal cord can be severed by forcing the tablespoon along the floor of the cranial cavity. The brain is then lifted out (fig. 2A) and transferred to a receptacle (fig. 3) which is provided for this purpose.

SUMMARY

1. The white mouse is the animal of choice in the laboratory diagnosis of rabies, Swiss mice being of no practical advantage.

2. Demonstration of Negri bodies in the test animal (white mouse) is the only dependable criterion for a positive diagnosis, since clinical manifestations are frequently too variable and indefinite to be of any value.

3. It is practical to attempt demonstration of Negri bodies by sacrificing mice on the eighth or ninth day after the inoculation.

4. The mouse-inoculation test is a more expedient means of diagnosis than the guinea pig method.

5. A simple procedure is described for demonstrating rabies virus in grossly decomposed animal brains.

6. Ether in final concentration of 10 per cent exerts a definite bactericidal effect upon contaminated dog brains after exposure for two hours at 4° C. This concentration has no effect upon the virulence of the rabies virus.

7. A simple technic is described for removing animal brains.

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Blockades of Progress

The tirades against rabies vaccination do not represent anything new in the fight against scientific medicine. They are expressions against the advancement of medicine as old as recorded history. We must live with them and break them down in dignified silence, for, in the voice from the Cross, "Forgive them, they know not what they do." The progress of medicine was retarded for many centuries that way. The burning of medical texts nearly 50 centuries ago and from time to time thereafter down to the raving of the *New York Sun et al* are merely flares of the same category that have kept the advance of medicine in abeyance but never won a victory. Mankind has always found the right way to protect itself against the whims of the misinformed, thanks to the minds of the thoughtful.

The nuclei of the atoms make up 99.25 per cent of the weight of all substances.

Hermaphroditism*

By JAMES McILVAINE PHILLIPS, M.D., B. J. BRIEF, T. S. SUTTON and JOHN WILLIAM MILLS, D.V.M.

Columbus, Ohio

THE ANATOMICAL manifestations of intersexuality, including those of both the internal and external genitalia, are evidently due to some heritable disturbance of the mechanism which governs the normal differentiation of the sexes.

These cases have been classified by Klebs,[1] and although his classification offers a convenient terminology which has been largely adhered to in reports of cases, it is more definite than is justifiable on the ground of morphology, since the malformations constitute a gradual series of combinations of variations between complete and abortive development of the derivatives of the Müllerian and Wolffian ducts. Many cases are borderline. It certainly has no basis from an etiological standpoint. In true hermaphrodites, when male and female gonads are present on both sides of the body, the condition is called bilateral hermaphroditism. When gonads of both sexes are present on only one side, the opposite side being one-sexed, the condition is termed unilateral hermaphroditism. When there is a male gonad on one side and a female gonad on the other, we speak of the condition as lateral hermaphroditism or gynandromorphism.

Case 2. External genitalia, with enlarged clitoris protruded.

PSEUDOHERMAPHRODITES

Pseudohermaphrodites also occur. Such individuals have gonads of one sex combined with the external genitalia and many of the internal or concealed parts of the genito-urinary apparatus which resemble more or less completely those of the opposite sex. From a clinical standpoint, Young[2] uses a simple nomenclature for these abnormalities. "If the gonads are testes," he states, "the patient is called a male pseudohermaphrodite; if ovaries, a female pseudohermaphrodite." Young's terminology will be followed in this report.

GENERAL CONSIDERATIONS OF INTERSEX

The term intersex is now used quite generally to designate both the true and pseudo hermaphrodite. Up to 1908, no less than 1,891 human intersexes, including several familial cases, were collected by Von Neugebauer.[3] Of the pseudohermaphrodites 795 were male and 246 were female. Perusal of the literature will show the frequent occurrence of intersexes in domestic animals.

An intersex horse, reported by Howell,[4] had two sibs with congenital, bilateral, inguinal hernia. This is interesting because inguinal hernia, often containing genital organs of the sex opposite to that of the external genitalia, is quite common in human cases. This horse was not examined microscopically. Two cases in cattle in which the diagnosis was confirmed by autopsy were reported by Curson.[5] A zebu with male pseudohermaphroditism was seen by Burt.[6] Fourie,[7] Brambell,[8] Davis

*From the departments of animal husbandry and veterinary medicine, Ohio State University.

and Pack,[9] and others described the condition in hogs. Tales of "morphodite" Angora goats are common among farmers, but such cases are usually found to have as their origin double cryptorchid animals. Cryptorchidism is frequent in this breed of goats and has been studied by Lush, Jones and Dameron.[10] Lush told the writer that in all of his experiments to determine the nature of cryptorchidism he had never seen a hermaphrodite Angora, unless one double cryptorchid with hypospadia could be considered as such. In Toggenburg and other milk goats it is common. Crew[11] described the different kinds of intersexes in 25 Toggenburg goats, two horses, one bull, one sheep, and one camel.

The only published report we have found of intersexuality in the dog is that of an English Cocker Spaniel observed by Hernaman-Johnson.[12] This case had one undescended testis and the external genitalia resembling those of a male, while autopsy showed a cystic gonad, a uterus and an imperfect vagina and other deviations from the normal.

We communicated with a large number of breeders whose combined experience would compass many thousands of individuals and found but three who had seen a hermaphrodite Cocker Spaniel. The three animals which these men observed were similar in external appearance and behavior to the two pseudohermaphrodites herein reported.

Cases of Familial Intersex Cited

Familial intersexes in man have been reported by many authors. We review some of these which are of particular interest because they demonstrate salient points.

Two siblings, which appeared to be females, with double inguinal hernias were operated upon by Davis,[13] who found a testis in each hernial sac. The diagnosis was confirmed by the microscope. No information as to other members of the family was given.

O'Farrell[14] reported on a family, with no ascertainable history of intersexuality in the previous generations, in which a number of intersexes appeared among the first cousins. There were three females and three males in this family. All of the females transmitted the defect. They had 19 normal children, and four were abnormal. One of the three males transmitted the defect. Eight of his children were normal and two were abnormal. They were probably of the male pseudohermaphrodite type, since the five cases which O'Farrell examined had very similar abnormalities, and those upon whom operations were performed had gonads which appeared to be testes on gross examination.

Mishell[15] reported on a family in which the father died at 67 following prostatectomy. The mother, 62 years of age, had diabetes. A maternal half-sister who had never menstruated was examined by a physician and found to have no uterus. Of eight living daughters one, married and childless, had infantile genitalia; another had hypomenorrhea, fat pads, and other evidences of pituitary disturbances; a third had uterine fibroids; two were apparently normal; and the other three were entirely feminine in external genitalia, figure, voice and hair distribution, but tubes and uterus could not be found. All three had bilateral inguinal hernias in which testis-like structures could be felt. Two were operated upon and male gonads were removed. Microscopic examination of these gave the typical picture of undescended testes. No trace of ovarian tissue was found by serial section. The third of these male pseudohermaphrodites was not operated. Estrogen was present in the urine of these patients, but it disappeared after operation and was replaced by gonadotropic substance.

The abnormalities found in a series of intergrade pigs that came from strains in which the condition was familial were described by Baker.[16] They showed a gradual range of variations between complete and abortive developments of male and female genital organs. The last pig in the series very closely resembled Mishell's human cases in that it had female external genitalia with no tubes or uterus while the gonads were testes. Some pigs in these families showed true hermaphroditism but the majority had testes as the only gonads. Crew[17] found familial male pseudoherma-

phroditism in goats. Their anatomical variations could also be arranged as a very definite series.

Human female pseudohermaphroditism, too, is familial. Two cases were cited by Polzer and Priezel[18] as occurring in the same family. No details as to siblings were given, except that there were five children in all.

Out of six siblings Rupilius[19] found two female pseudohermaphrodites with external genitalia resembling male organs, without testes, but with uterus and ovaries. He gave a review as of date of the literature of familial hermaphroditism in man.

Cases 10 and 11 reported in Young's monograph were sibling female pseudohermaphrodites with identical findings, *viz.:* 1) Greatly enlarged adrenals with hyperplasia of the androgenic, or prenatal zone of the cortex; 2) hypoplastic or infantile ovaries with multiple cysts; and 3) an enlarged clitoris, a persistent urogenital sinus into which the vagina opened, and, surrounding the vaginal orifice, a hymen.

Young also stated:

> Recently, I have seen in consultation in another city two patients who presented all the characteristics of female pseudohermaphrodites * * *. These patients were siblings, one 16 and the other about ten years younger.

Human familial intersexes seem to be divided into two groups. In the first group the abnormal offspring are either male pseudohermaphrodites or true hermaphrodites; in the second all abnormal offspring are female pseudohermaphrodites.

In domestic animals both male pseudohermaphrodites and true hermaphrodites occur in the same families and are evidently of similar genetic origin.

We have been unable to find a single report of female pseudohermaphroditism in any domestic animal, the only mammalian instance being that of an intersex opossum described as a reciprocal freemartin by Hartman and League.[20]

FREEMARTINS

Another variety of intersex, called freemartin, is common in cattle. This condition is acquired, not zygotic. Lillie[21] showed that this variety of bovine intersex is a zygotic female and is always born with a normal bull twin. He attributes its intersexual structure to the effect of the male sex hormones which, circulating in its blood as a result of anastomosis of allantoic vessels, inhibit in varying degrees the development of the ovaries and female internal genitalia and stimulate the development of testicular tissue and the derivatives of the Wolffian system. The external genitalia are but slightly affected and remain female in type.

Chapin,[22] from a histological study of freemartin embyros, concluded:

> As a result of the introduction of the interstitial secretion of the male, those organs in the freemartin which are present in the indifferent stage develop toward the male condition (rete, first set of sex cords, primary albuginea), and those which develop in the normal female at sex differentiation or later are inhibited from developing (cords of Pflüger, definitive albuginea, union of Müllerian ducts to form uterus).
>
> The high degree of variation found in the organs of the reproductive system of freemartins is indicative of the variability of the time at which the interstitial secretion of the male embryo may first be introduced into the circulation of the female embryo, and the amount which may be introduced.

Baker suggested that the male hormones are more powerful than the female, and that this may be the answer to Lillie's question as to why bull calves are not affected by their mothers' hormones.

It is difficult to distinguish between some of the intersex modifications of structure which are found in single births and those found in some of the most extreme modifications of freemartins. Indeed, it is highly probable that some of the cases of bovine intersexes which have been reported are, in origin, freemartins, and that some of the cases reported as an extreme development of the freemartin condition are examples of zygotic intersexes.

Although cattle are the only animals in which anastomosis of the fetal circulation of dizygotic twins is common, cases of such vascular anastomosis in goats[23] and of freemartins in sheep[24] have been described. Hence, all intersex cases in ungulates should be critically examined. Lillie[21] called at-

tention to the possibility of intersexes' originating as freemartins and being born as apparently single individuals, owing to the male twin having perished early in its development. Again it is perfectly possible for an intersex to be born with a brother which had not influenced its abnormality, as in the human case reported by Neumann.[25] This intersex had a partially descended right testis and an ovatestis on the left, and a uterus and tubes; there was a penis with a meatus. In freemartins the external genitalia are typically feminine, usually without enlargement of the clitoris, and the Müllerian structures and ovarian tissue are largely suppressed; therefore, it would seem certain that this intersex must have been coincident with, rather than caused by, the male twin.

A diagnosis between a zygotic intersex and a freemartin can not be made on morphology alone, as illustrated by the familial cases of Mishell,[15] which showed a very close approximation to the morphology of the freemartin in that there was no evidence of uterus, tubes, or ovarian tissue, and the gonads resembled testes while the external genitalia were female with the normal clitoris. Nevertheless, there was no suggestion of these subjects' being freemartins. They occurred as three single births. There is no evidence to show that freemartins occur in the human being.

Among the pig intersexes described by Baker,[16] the anatomy of one individual closely resembled that of a freemartin excepting for the exaggerated clitoris. Several cases of two pig embryos with fused chorionic circulation, one of each pair a male and the other an intergrade, were described by Hughes,[26] who considers them freemartins.

The freemartin and all other varieties of intersexes caused by hormone action other than that elaborated within the embryo itself are classified by Crew[11] as "intersexuality due to the overriding of the genotype."

There are a large group of intersexes which are not freemartins and must originate as a result of some other condition which must be zygotic, since it is familial. This includes all mammalian intersexes which possess testicular tissue, excepting the freemartins.

CREW'S HYPOTHESIS OF ZYGOTIC INTERSEX

The best known hypothesis on the occurrence of these intersexes of the male pseudohermaphrodite and true hermaphrodite types is that of Crew,[17] who starts with the premise that in embryos, up to a certain stage, male and female are alike and that the genital primordia require stimulation or inhibition at critical and definite times in the course of their differentiation in order that normal male and female organs may be the end product. He believes that the male and female hormones elaborated by specific interstitial cells of the gonads perform this task. The male hormone instigates the development of the Wolffian and the female that of the Müllerian ducts, and germinal epithelium is stimulated to form testes in one sex and ovaries in the other. A retardation in the production of such stimuli or an insufficient amount or impairment in the quality of them, Crew believes, is the cause of malformations, and he considers that "this retardation insufficiency of the sex hormone is a character in the genetic sense, and classifies these male pseudohermaphrodites as "A, Intersexuality due to abnormalities in the time of the differentiation of the gonads."

Closely allied to class A is another group of cases which have ovarian tissue in addition to the testicular (true hermaphrodites). These he classifies as "B, Intersexuality due to abnormality in the mode of differentiation of the gonad." The ovarian tissue may be either an ovary or an ovatestis, some animals having two complete sets of gonads. Crew thinks that both of these types probably originate as zygotic males and differ from each other only in the time and mode of differentiation of the gonad. Baker suggests that both types may begin as zygotic females in which, due to two inherited recessive factors, one of which is sex-linked, the male hormones are produced, and cause partial reversal of sex.

ANATOMICAL FEATURES

It is probable that the hormones of the male gonads vary greatly in quality and

quantity at different periods during ontogeny. Whitehead[27] has shown that the histological picture changes at definite periods. In view of the experimental work of Moore,[28] it is not surprising to find fairly perfect ovarian and testicular tissue in the same adult animal, but the question of why one did not suppress the other during ontogeny is still open to a satisfactory answer backed by experimental evidence.

In mammalian hermaphrodites the ovarian tissue often produces ova, and discharge of ova with evidence of a modified heat cycle occurs. The testicles, however, are much less complete; degeneration of the tubules is common; and the occurrence of perfect spermatogenesis even when the testicle has partially descended has not been recorded. The testicular structure in all intersexes is like that of a cryptorchid with the same hyperplasia of the interstitial cells. This is also true of the male pseudohermaphrodite. In view of the imperfect spermatogenesis in partially descended intersex pigs' testicles,[29] where the scrotum is naturally sessile and the temperature of the testis can not vary much from normal, one doubts that the higher temperature[30] of abdominal testicles is a complete explanation for the lack of development in the male gonads of an intersex.

When there is an ovatestis, a narrow band of connective tissue usually separates the elements; but, if they are completely separated, the testicle usually has partially descended. Some human cases may have scrotal ovaries, as in Wolf's case.[31]

It is difficult to account for the bizarre arrangement of structures in cases of hermaphroditismus lateralis verus as a result of ill-timed or deficient hormonic stimulation alone. Cases have been reported in man by Young,[2] in the guinea pig by Jaffe and Papanicolaou,[32] and in hogs by Kadletz,[33] Corner, [34] Crew, and Baker. Some authors consider them as true bilateral gynandromorphic mosaics. The cases with an ovatestis and various other combinations of the gonads are regarded by them as examples of an irregular mosaic pattern. But, true gynandromorphic mosaics can not occur in mammals, where the control of the processes of sex differentiation is due to the elaboration of endocrines by the developing gonads.[35] However, all intersexual forms from male pseudohermaphroditism to bilateral true hermaphroditism have been found in the same strain of animals, in the goat and pig by Crew, and in the pig by Baker. More than one form may be in the same litter of pigs; hence, they must have a common genetic origin and are probably due to multiple recessive factors.

In intersex pigs which have a testicle on one side of the body and an ovary or ovatestis on the other, the testis is invariably the right gonad, while the left is ovary or ovatestis, in which case the ovarian tissue is always anterior to the testicular, often sitting on it cap-wise. Crew thinks that this indicates that gonadic differentiation is not synchronous but consecutive and proceeds from the caudad to the cephalad pole, and that the formation of both kinds of tissue is due to alternating action of the sex-determining factors. As already stated, goat and pig intersexes have essentially female external genitalia, the chief deviation being an enlarged clitoris. But in man the external genitalia of true hermaphrodites may be either a mixture of male and female structures; entirely female in character; or male with a true penis, prepuce, and meatus, a normal perineum, with no trace of vagina or labia, and a poorly developed scrotum. In such cases the uterus usually opens into the male urethra. It will be noted that the canine hermaphrodite reported in this paper has male external genitalia, while the two male pseudohermaphrodites have external genitalia resembling those of a female, excepting for the penis-like clitoris.

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Male pseudohermaphroditism and true

tention to the possibility of intersexes' originating as freemartins and being born as apparently single individuals, owing to the male twin having perished early in its development. Again it is perfectly possible for an intersex to be born with a brother which had not influenced its abnormality, as in the human case reported by Neumann.[25] This intersex had a partially descended right testis and an ovatestis on the left, and a uterus and tubes; there was a penis with a meatus. In freemartins the external genitalia are typically feminine, usually without enlargement of the clitoris, and the Müllerian structures and ovarian tissue are largely suppressed; therefore, it would seem certain that this intersex must have been coincident with, rather than caused by, the male twin.

A diagnosis between a zygotic intersex and a freemartin can not be made on morphology alone, as illustrated by the familial cases of Mishell,[15] which showed a very close approximation to the morphology of the freemartin in that there was no evidence of uterus, tubes, or ovarian tissue, and the gonads resembled testes while the external genitalia were female with the normal clitoris. Nevertheless, there was no suggestion of these subjects' being freemartins. They occurred as three single births. There is no evidence to show that freemartins occur in the human being.

Among the pig intersexes described by Baker,[16] the anatomy of one individual closely resembled that of a freemartin excepting for the exaggerated clitoris. Several cases of two pig embryos with fused chorionic circulation, one of each pair a male and the other an intergrade, were described by Hughes,[26] who considers them freemartins.

The freemartin and all other varieties of intersexes caused by hormone action other than that elaborated within the embryo itself are classified by Crew[11] as "intersexuality due to the overriding of the genotype."

There are a large group of intersexes which are not freemartins and must originate as a result of some other condition which must be zygotic, since it is familial. This includes all mammalian intersexes which possess testicular tissue, excepting the freemartins.

CREW'S HYPOTHESIS OF ZYGOTIC INTERSEX

The best known hypothesis on the occurrence of these intersexes of the male pseudohermaphrodite and true hermaphrodite types is that of Crew,[17] who starts with the premise that in embryos, up to a certain stage, male and female are alike and that the genital primordia require stimulation or inhibition at critical and definite times in the course of their differentiation in order that normal male and female organs may be the end product. He believes that the male and female hormones elaborated by specific interstitial cells of the gonads perform this task. The male hormone instigates the development of the Wolffian and the female that of the Müllerian ducts, and germinal epithelium is stimulated to form testes in one sex and ovaries in the other. A retardation in the production of such stimuli or an insufficient amount or impairment in the quality of them, Crew believes, is the cause of malformations, and he considers that "this retardation insufficiency of the sex hormone is a character in the genetic sense, and classifies these male pseudohermaphrodites as "A, Intersexuality due to abnormalities in the time of the differentiation of the gonads."

Closely allied to class A is another group of cases which have ovarian tissue in addition to the testicular (true hermaphrodites). These he classifies as "B, Intersexuality due to abnormality in the mode of differentiation of the gonad." The ovarian tissue may be either an ovary or an ovatestis, some animals having two complete sets of gonads. Crew thinks that both of these types probably originate as zygotic males and differ from each other only in the time and mode of differentiation of the gonad. Baker suggests that both types may begin as zygotic females in which, due to two inherited recessive factors, one of which is sex-linked, the male hormones are produced, and cause partial reversal of sex.

ANATOMICAL FEATURES

It is probable that the hormones of the male gonads vary greatly in quality and

quantity at different periods during ontogeny. Whitehead[27] has shown that the histological picture changes at definite periods. In view of the experimental work of Moore,[28] it is not surprising to find fairly perfect ovarian and testicular tissue in the same adult animal, but the question of why one did not suppress the other during ontogeny is still open to a satisfactory answer backed by experimental evidence.

In mammalian hermaphrodites the ovarian tissue often produces ova, and discharge of ova with evidence of a modified heat cycle occurs. The testicles, however, are much less complete; degeneration of the tubules is common; and the occurrence of perfect spermatogenesis even when the testicle has partially descended has not been recorded. The testicular structure in all intersexes is like that of a cryptorchid with the same hyperplasia of the interstitial cells. This is also true of the male pseudohermaphrodite. In view of the imperfect spermatogenesis in partially descended intersex pigs' testicles,[29] where the scrotum is naturally sessile and the temperature of the testis can not vary much from normal, one doubts that the higher temperature[30] of abdominal testicles is a complete explanation for the lack of development in the male gonads of an intersex.

When there is an ovatestis, a narrow band of connective tissue usually separates the elements; but, if they are completely separated, the testicle usually has partially descended. Some human cases may have scrotal ovaries, as in Wolf's case.[31]

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hermaphroditism seem to have a common chromosomal origin and to differ from each other only in the degree of their abnormality. These types occur in both animals and man. They are transmitted by apparently normal parents and come through both the male and female lines. Since the abnormalities occur in a gradual series with no sharp line of demarcation between them, beginning with double cryptorchidism with hypospadia and ending with complete bilateral hermaphroditism, they are probably due to multiple recessive factors.

Goldschmidt[36] considers intersexes as individuals, with the chromosomal makeup of either male or female, which actually develop as such up to a certain instant which he calls the turning point (*drepunkt*), and from then on develop in the opposite direction. According to whether the turning point is earlier or later, the intersex will be a more or less pronounced admixture of the two sets of sex characters, although each of its cells, genetically, is of the original sex. He thinks that intersexuality is due to a mutation of the valence of the genes, and has produced many intersexual forms by interracial breeding of insects.

Crew thinks that the intersexual conditions found in goats may also have their origin in interracial crossing, as in Goldschmidt's Lymantria. Baker, who studied the inheritance of intersexuality in England and in the New Hebrides, where intergrade pigs are highly prized for their use in religious rites and are intentionally bred by the natives, found that more sows transmitted the defect than did boars and that the ratio of abnormal to normal young in affected litters was 1:8.

In Saanen milk goats 20.2 per cent of the offspring from matings of carrier by carrier were hermaphrodites, while in the herds of Toggenburgs reported by Eaton and Simmons,[37] who considered this defect a single recessive character, the percentage was 22.1.

Many other theories have been advanced as to the nature of the intersex. Most of them pay more attention to reciting the order of their embryological development than to attempting to explain the chromosomal factors involved, but numerous hypotheses have been suggested as to the nature of the genes. All agree that they are recessives but the number of factors involved, the mode of their inheritance, and their linkage with or limitation by sex have not been established.

Whether these deformities are due entirely to heritable irregularities in the structures which elaborate the sex hormones or whether they also have associated with them alterations of certain somal genes which normally direct the cells as they arrange themselves to form some of the sex organs is still an algebraic x as well as a chromosomal xy.

The experimental clinical use of various endocrine substances in human cases of organic sex abnormalities as well as research in experimental biology have resulted in a vast accumulation of reports on the effects of the sex hormones in man and in developing animal embryos. Many of the details seem to be contradictory but they will probably be clarified as our knowledge increases and is correlated. However, it seems clear that irregular sex hormones have a great influence on the development of abnormal sex organs.

Human female pseudohermaphroditism is probably due to masculinization of females as a result of hyperplasia of their adrenal cortex or to the presence of supernumerary adrenals, and in some cases to tumors originating in the adrenals or other structures which elaborate similar androgenic hormones.[38] The fact that the ovarian medulla, the adrenal cortex, and the testicle in mammals have a common embryonal origin suggests an explanation of this.[39] As an example of masculinizing tumors, Kline[40] called attention to the effects of arrhenoblastomas. Surgical removal of such tumors may be followed by refeminization and even by pregnancies and the birth of children.[41] Hirsutism and other male characters, such as atrophy of the mammae, coarse voice, and hypertrophy of the clitoris, known as Apert's syndrome,[42] may be found in women with ovarian cysts. Abnormal ovaries cause the doe deer to produce horns[43] and the cow to become bullish.[44]

The masculine alterations of the external

genitalia of the zygotic female embryo seem to be most marked when the abnormal male hormone-producing tissue is present in early embryonal life. We find a continuous series beginning with the female, who has been born sexually normal and later has assumed male characters, and ending with an intersex with hypoplastic ovaries and external genitalia resembling those of the male, and with various modifications of the internal genitalia in the male direction—the female pseudohermaphrodite.

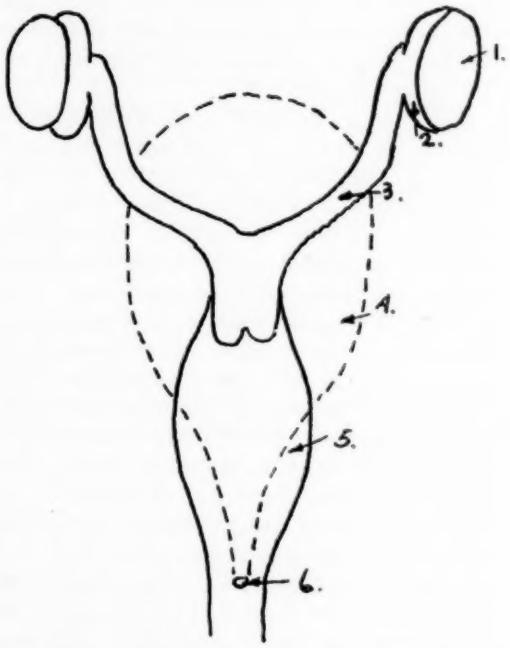
In zygotic males the evidence seems to show that the same abnormalities of the adrenals cause some types of hypervirilism, but the familial relation of these to female pseudohermaphroditism has not been established.

Adrenal rests or bilateral hyperplasia of the adrenal gland is found in at least 15 per cent of female but only 0.7 per cent of male pseudohermaphrodites, according to Glynn.

Female pseudohermaphroditism associated with hyperplasia of the adrenal cortex in humans is apparently a heritable entity, since several cases in siblings in addition to those we have cited appear in the literature. They apparently do not occur in the same families with male pseudo or true hermaphrodites, and are far less common. In animals we find no record of female pseudohermaphrodites. These facts would seem to preclude the possibility that the different forms of intersexuality are due to occasional crossing over of genes on the sex chromosomes.

As a general rule, fully formed adult structures are much less susceptible to modification by endocrines than are developing embryonal tissues. Masculinization of women in later life offers an exception. Neumann[45] reported a case of extreme masculinization of a woman due to a testicular adenoma of the ovary. The clitoris assumed penis-like proportions and other structures were profoundly altered. Surgical intervention caused the evidences of sex reversal to disappear and the woman subsequently became pregnant.

In masculinization of women due to the various tumors of the ovary, the patients become quite normal in a short time after the removal of the tumor, with the exception of the clitoris, which usually remains enlarged.[46] The marked contrast between these cases and the freemartin with its female external genitalia shows how much there is to learn about sex hormones and their effects. Many authors cite evidence to show that tissues which are masculine in their chromosome formula respond most readily to the male hormones and that the converse is also true. While this may solve some of the enigmas, it presents other problems.



Case 2. Diagram of internal genitalia. (1. Gonad. 2. Epididymis. 3. Uterine horn. 4. Bladder. 5. Vagina. 6. Urogenital sinus.)

Notwithstanding the numerous reports of human familial female pseudohermaphroditism, we have seen no discussion of it as a heritable character in the genetic sense.

In the human male pseudohermaphrodite or true hermaphrodite the general behavior, libido, and emotional complex may be masculine in some individuals and feminine in others. Some patients can not be definitely classified, since their behavior is as chaotic as their anatomy. The relationship of their environmental influence to their mental attitude would make an interesting psycho-

logical study. That environment is not entirely responsible is well illustrated by the case of a child raised as a girl in wholesome surroundings who escaped the parental rooftree as an adult and became a hard-drinking, profane, and capable horse jockey. As a contrast, Rubovitz and Saphir[17] reported a case in which the patient, reared as a girl, had testes as the only gonads. There were no developed internal female organs but the external genitalia were female with an enlarged clitoris, a vagina, and labia, in one of which there was a testis. This patient had many physical characteristics resembling the male, but her general behavior was feminine, and her libido extremely so.

The plastic surgeon has a difficult problem to solve when he attempts to decide whether it is best to transform a given individual into a cosmetic male or female, in order to improve its morale. The nature of the gonads alone is not a sufficient guide as to what is best for the patient.

In animals the male pseudohermaphrodite behaves as a male, and his secondary sex characters are also masculine. True hermaphrodites are very similar, but a few individuals may undergo what appear to be typical heat cycles, at which times their general behavior is that of a female.

The following case histories with their necropsy reports will serve to show how similar the anatomy of the male pseudohermaphrodite dog is to that of the same type of abnormality in the pig, goat, and man. Cases 1 and 2 came from the same sire. This dog produced many litters of puppies with no other intersexes appearing among them. Although there have been several matings between his sons and daughters, we have not succeeded in obtaining other intersexes.

CASE 1

The dam of case 1 produced three other litters which were normal. In this litter the sibs were two normal males, one female which has proved by three matings to be sterile, and the intersex which we are reporting. This animal was supposed to be a female but its extremely masculine behavior when its mother came in season caused the owner to examine it carefully. He had noticed that it assumed the male attitude when urinating. It was killed and Dr. Pearson of Findlay, Ohio, made the following notes of the postmortem examination.

The subject is a black Cocker Spaniel, about 6 months old. The external genitalia appear like those of a normal bitch, except that the vulvar tip is a little longer than normal, and it has a number of stiff hairs on its point. Just showing through the slightly separated labia is the rather red, conical tip of an exaggerated clitoris, which can be extended by pressure to about 2 inches. A nodular structure can be palpated in the right labia. The clitoris contains a bone and is curved like a sicle with its point upward. It is covered with a smooth, shiny mucous membrane and resembles a normal dog's penis in general shape but contains no urethra. Its base is attached to the pubic arch. The meatus is located at the base of the clitoris, and anterior to it, with its opening well inside the labia.

On opening the abdomen nothing abnormal is noted in the bladder, kidneys, or adrenals. In the position normal for the left ovary is an oval mass which looks like a testicle. It has an epididymis which is slightly separated from the gonad, but which is about the same length. The gonad is joined to a round ligament which extends through the inguinal canal. An expanded uterine horn is tightly bound to it. The horn is also attached to the round ligament. On the right side the general arrangement is the same, except for the position of the gonad, which is in the inguinal canal. Its epididymis is larger and closely approximated to the gonad. Both gonads have vascular and nerve supplies. The uterine cornua do not have fimbria but are slightly extended into a cup and attached firmly to the gonad. The underdeveloped uterine horns join to form a corpus which is a little smaller than one normal for this size of animal. There is a definite cervix. The pouch behind the uterus is very deep. The vagina is well developed. The mammary glands are male in character, and there is no nodule in the position occupied by a normal male prepuce.

Dr. Pearson kindly permitted us to take sections from the gonads of the specimen, which, on microscopic examination, gave a picture identical with that found in undescended testes of the cryptorchid and with that of the testes of pseudohermaphrodites; that is, degeneration of the tubules, absence of spermatogenesis, and a hyperplasia of the interstitial cells. It should be noted that the partially descended testis of

this specimen did not show spermatogenesis even though it was plainly palpable.

CASE 2

This dog was obtained from its owner when it was about 9 months old. It, too, was thought to be a female before its male posture when urinating was noted. This and its tendency to fight male dogs led to a disclosure of its abnormality. It was entirely male in behavior when near bitches in season. Its sibs were one normal male and one fertile female. Due to dystocia the mother was delivered by cesarian section and ovariectomized. Consequently, this was not only her first litter but it was also her last. The autopsy notes follow.

The subject is an adult black Cocker Spaniel. In the usual position there are two apparently normal labia in which nothing but fat can be palpated. The clitoris is much exaggerated and, on palpation, 2.5 cm. can be disclosed; also, it contains a bone. There are no openings on the surface of the clitoris. Immediately ventral to this organ is located the opening of the urogenital sinus. A large tuft of long hairs occurring at the anterior borders of the labia bears a striking resemblance to the tuft of hairs found about the prepuce of the male. The teats are small and resemble those of the male.

Upon opening the abdomen two gonads are found in the positions normally occupied by the ovaries. The right is a roughly ovoid structure 1.5x1.0x0.7 cm.; its surface is smooth and invested in peritoneum which closely binds it to an epididymis-like structure which covers one margin of the gonad from pole to pole in a C-shaped manner. It extends beyond the gonad and is 2.3 cm. long. The left gonad is 1.5x1.5x1.0 cm. and its surface is marked with tiny swellings. It is separated from its epididymis by a peritoneum-like sinus epididymis. Arteries, veins and nerve trunks join each epididymis and supply the gonads. A "round ligament" extends from the lateral aspect of each gonad through the inguinal canal where it terminates in the subcutaneous fascia. They do not extend into the labial swellings.

Two apparently normal uterine horns terminate at and are closely fused with the gonad by much connective tissue at their anterior poles, where gonad and epididymis join. No fallopian tubes or fimbria are seen. The horns appear normal but measure only 7.5 cm. in length and 0.5 cm. in diameter. They are attached to broad ligaments, and each gonad with its uterine horn is also attached to the great omentum.

The posterior parts of the uterine horns are fused to form a uterine body 1.5 cm. in length which terminates in a well-defined cervix. The retrouterine pouch is deep. The cervix extends into a well-defined vagina 5 cm. in length and 1 cm. in diameter. The uterus and bladder have their normal relative positions.

The urethra and the vagina join to form a urogenital sinus. The opening into the sinus is a narrow slit situated between two slight elevations. The sinus is invested on each side by a large, gland-like mass, consisting of sheaths of muscles surrounding large complexes of blood sinuses. These corpora cavernosa are located about 5 cm. internal to the external opening of the sinus. The sinus opens externally immediately behind the clitoris and this surface of the clitoris is smooth. The other surfaces are rough and covered by papillae. Two membranous folds limit the urogenital sinus laterally in the last centimeter of its extent and they serve to attach the clitoris to the skin of the labia. The ischiocavernosus muscle binds the clitoris to the pubic arch.

There is no mammary gland tissue. The labia contain only fat and fascia. The adrenals do not appear enlarged.

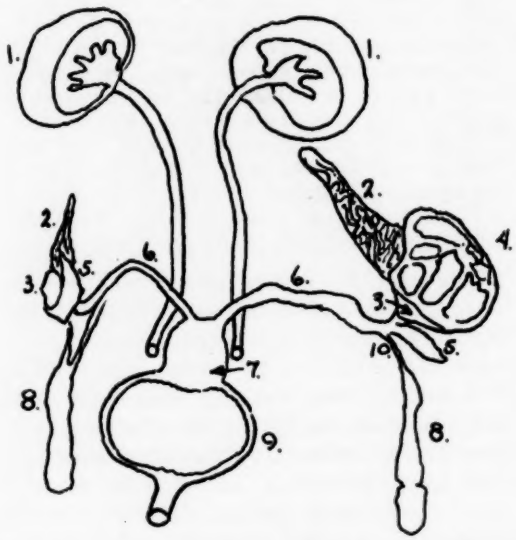
HISTOLOGICAL EXAMINATION—CASE 2

The gonads and accessory sex organs were sectioned and stained by Masson's trichrome technic. The gonads presented the appearance of typical cryptorchid testes. The seminiferous tubules were lined with tall, columnar epithelium in which no active mitosis was evident. This is the condition seen in the germinal epithelium of the ordinary cryptorchid testis. The interstitial tissue was present in large quantities and the individual cells appeared to be hypertrophied. In the connective tissue septa of the gonad, two types of interstitial cells appeared. Both types lay in columns between the connective tissue fibers. The one type appeared to be identical morphologically and in staining characteristics with the ordinary interstitial cells. The other type, however, showed a clear cytoplasm in contrast to the moderate granularity of the first. These cells closely resembled paralutein cells.

The tubules of the epididymis were lined with columnar epithelium bearing stereocilia. Occasionally, they showed some cell débris, but no evidence of spermatogenesis could be found. The epididymis also contained many lymphoid follicles. Neither primitive ovarian follicles nor typical ovarian stroma could be found in either gonad or epididymis. The uterine horns

were lined with typical uterine epithelium, bearing normal but inactive uterine glands. Fused to the uterus was another tube which was invisible macroscopically. Its lumen was smaller than that of the uterine horns and was lined with a simple cuboidal epithelium. This tube was evidently the Wolffian duct, since it could be traced through serial sections into the epididymis.

The vagina was lined with simple cuboidal epithelium. The thyroid gland appeared normal. The hypophysis showed some eosinophilia and the pars nervosa was probably enlarged.



Case 3. Diagram of internal genitalia. (1. Kidneys. 2. Plexus of blood vessels. 3. Testes. 4. Ovary. 5. Epididymis. 6. Uterine horns. 7. Uterus. 8. Wolffian duct. 9. Prostate. 10. Müllerian end duct.)

CASE 3

A dark-brown Irish Setter-like dog, 10 years old, which appeared to be a normal male excepting for a partially developed scrotum, was brought to the clinic of the Ohio State University to be destroyed because male dogs were attracted to it about twice a year. This became more annoying and persistent as the dog grew older. There had been no opportunity to observe its reaction to bitches in season. The following observations were made at autopsy.*

*From the laboratory of veterinary pathology, Ohio State University.

The subject is a brown setter dog weighing about 40 pounds. The external genitalia are those of a normal male excepting that in the position normally occupied by the scrotum there is but a slight cutaneous pouch. The teats are like those of a normal male dog.

Upon opening the abdomen, the gonads are found attached to broad ligaments in the position usually occupied by the ovaries. On the left side the gonad is divided into two distinct portions. The anterior and lateral part is the larger. It is ovoid in shape and is 7x6x4.5 cm. The surface is irregular and covered by yellowish nodules. The other portion of the gonad, posterior and medial in position, is triangular in shape and measures 4.5x1.5x1.0 cm. A cone-shaped plexus of blood vessels 8.5 cm. in length extends from the gonad toward the posterior pole of the kidney. A small uterine horn terminates at its anterior pole. The right gonad is smaller, roughly ovoid, and flat. There is a rather deep fissure in the center which almost divides the gonad. The lateral portion is flat and ovoid and measures 2.0x1.2x1.0 cm. A cone-shaped plexus of blood vessels 4 cm. in length extends from it to the kidney. The medial portion of the gonad is rectangular, with dimensions of 3.5x1.0x1.0 cm. A small uterine horn terminates at its anterior pole. A uterus is found in the normal position. Its body is pear-shaped and measures 4.0x3.2x2.5 cm. Small horns extend from it to the gonads. It is connected with the urethra by a small canal which passes through a prostate gland, which measures 7.0x7.0x4.8 cm.

HISTOLOGICAL EXAMINATION—CASE 3

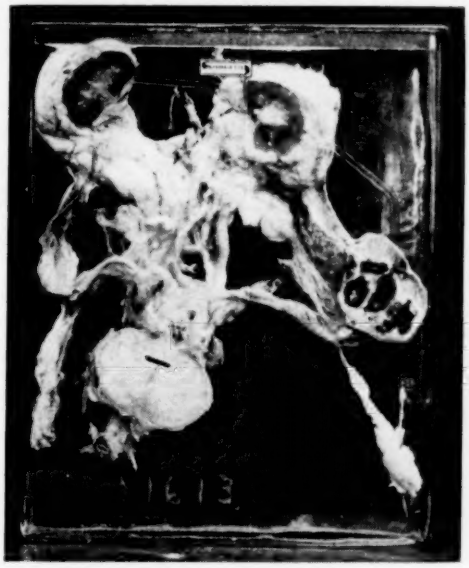
The gonads and accessory organs were sectioned and stained with Delafield's hematoxalin and eosin. The right gonad was covered with dense, regular connective tissue (= albuginea). Extending inward from this dense wall were numerous septa of less dense connective tissue. The seminiferous tubules were lined with columnal epithelium in which no active mitosis was found. Leydig cells were present in large quantities around the seminiferous tubules. The cells were irregular as to shape; some were large and spherical with dark-staining granules, and others were elongated or spindle-like. The tubules of the epididymis were lined with columnar epithelium. No evidence of spermatogenesis could be found. The left gonad contained a number of large, cyst-like structures which were filled with a clear fluid. They were outlined by a rather

dense connective tissue wall. Next to this was a layer of dark-staining round and ovoid cells which appeared like columnar cells (= granulosa). There was much variation in the thickness of the layer. The fluid which coagulated on fixing appeared granular and took a pink stain. The nodules, which appeared yellowish grossly, had a loose connective tissue network interlaced with ovoid epithelioid cells, with dark-staining nuclei and granular cytoplasm. There was a well-developed network of large, thin-walled capillaries. The structure was suggestive of corpus luteum. The microscopic picture of the cystic portion of this gonad exactly resembled that of cystic ovaries of old bitches, excepting for the greater number of granular cells present in this specimen. On the medial side of the gonad and extending inward to 1-cm. depth and in loose connective tissue were seminiferous tubules. They were lined with columnar epithelium, in which no active mitosis was found. The lumen of the tubules was filled with cell débris. The interstitial tissue appeared the same as that described in the right gonad.

Medial to the gonad and loosely attached was the epididymis. The tubules were lined with columnar epithelium. The uterine horns were lined with typical uterine epithelium with inactive glands. Very little could be told about the uterus, due to the presence of purulent inflammation. The small canal which connected the uterus with the urethra was lined with stratified squamous epithelium.

The prostate gland showed marked hyperplasia with areas of purulent inflammation. The acini were lined with stratified squamous epithelium. The thyroid glands also were greatly enlarged.

Although no normal Graffian follicles were found in the left gonad, this organ was probably an ovatestis with the ovarian portion greatly modified by cystic degeneration, which is not surprising in view of the age of the dog. Therefore, this animal can be classed as a case of unilateral hermaphroditism. The gross anatomy of this specimen closely resembled that found by Hernaman-Johnson[12] in his English Cocker hermaphrodite.



Case 3. Photograph of internal genitalia.

CONCLUSIONS

Male pseudohermaphroditism occurs in dogs and is probably familial, although the numbers found are insufficient to establish this definitely.

In a male pseudohermaphrodite with one testicle in the labium, where the temperature must have approached that of the normal testicle, spermatogenesis was absent.

Unilateral hermaphroditism occurs in dogs, and the anatomical abnormalities in cases reported are quite similar to those met with in some human cases but differ from those seen in other domestic animals in that the external genitalia are masculine in character with no evidence of vulvar structures.

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Paravitaminoses

By paravitaminosis is meant the pathological state resulting from a vitamin privation; in other words, the disease developed beyond the stages of response to the administration of the deficient factor. It is to a common avitaminosis what paresis and locomotor ataxia are to syphilis. Structures have been damaged by the invasion of bacteria or degenerative changes which can not yield to vitamin treatment. Blacktongue is a good example in animals. When the course of the disease has reached the advanced stage, nicotinic acid is no longer effective, no more so than arsphenamines in tabes dorsalis. Paravitaminoses open up a broad field for investigation. They extend the study of etiology far beyond the appearance of clinical symptoms.

Hydrooxyethyl aprocupreine is the name of a new drug that is said to cut the mortality of pneumonia (human) in half. It is a product of the Mellon Institute of Pittsburgh.

Sodium Chlorate Poisoning*

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SINCE the general acceptance of sodium chlorate as a herbicide some ten years ago, large quantities have been used to control or eradicate noxious plants. On many farms animals have had access to the sodium chlorate-treated plants which, because of their markedly salty flavor, are very palatable to animals. Since the number of farm animals known to have been poisoned by sodium chlorate is relatively small, perhaps due in part to the failure of veterinarians to diagnose all cases of such poisoning, those in charge of weed control work have come to regard sodium chlorate as presenting little hazard to farm animals. The writers have observed horses which suffered no apparent ill effects after closely grazing areas of heavily sprayed bindweed, *Convolvulus arvensis* L., also called wild morning glory. The spray used consisted of 1 pound of sodium chlorate dissolved in each gallon of water and had been applied at the rate of 4 gallons per square rod.

The results of numerous trials show that live stock can consume moderate amounts of treated weeds without harm and this has, in a few instances, led to careless methods of handling. Sodium chlorate, $NaClO_3$, is a powerful, although moderately slow-acting, oxidizing agent. It is possible, therefore, that the residual material consumed by animals which eat sodium chlorate-sprayed plants consists largely of sodium chloride—common salt. The danger of sodium chlorate poisoning from consuming sprayed plants would therefore decrease rapidly as the chemical combined with the plant tissues, and the greatest hazard would be immediately following spraying. In case the action of the sodium chlorate upon certain plant constituents should result in the formation of toxic compounds or the treatment should render certain poisonous but unpalatable plants attractive to the animals, the peril might persist for a considerable period.

The probability of treated soil remaining dangerous appears remote, as Harper[1] found that in a moist soil at summer temperatures, the sodium chlorate was decomposed in less than seven days. While it is known that sodium chlorate may be absorbed by both roots and rhizomes, it is doubtful whether these would acquire a sufficient concentration of the chemical to be toxic.

The danger of fire from sodium chlorate—organic material mixtures should not escape emphasis. Cook[2] found that concentrations greater than 10 per cent of the chemical with organic materials constituted a definite fire hazard at relative humidities below 75 per cent.

Review of Literature

Frohner[3] found that single doses of 25 Gm. of potassium chlorate had no effect on a wether, while 50 Gm. caused inappetence, cessation of rumination, and a transient depression. Horses withstood 30 and 40 Gm. without visible symptoms. A cow was given 50 Gm. and, then, two days later, 100 Gm., with no apparent ill effects. Zimmermann, quoted by Frohner, gave the following amounts as fatal doses: Horse, 250 Gm.; cow, 500 Gm., sheep, 100 Gm.; dog, 60 Gm. Since the molecular weight of potassium chlorate is 122 and sodium chlorate 106, the above figures should be reduced by approximately one-eighth to be applicable to sodium chlorate.

Gildow[4] found that a total of 3 ounces of sodium chlorate or calcium chlorate, given in two equal doses four hours apart, was sufficient to kill yearling wethers weighing approximately 100 pounds each. Fitch, Boyd and Hewitt[5] produced severe symptoms of poisoning and abortion in a cow

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by the oral administration of a total of 400 Gm. of sodium chlorate over five successive days, while 180 Gm. over a period of three days produced such severe symptoms in another cow that the experiment was discontinued.

Seddon and McGrath[6] killed sheep within 24 hours by the oral administration of 60 to 90 Gm. (2 to 3 ounces). To test both the palatability and toxicity to bovines, a mixture of one part bone meal and two parts sodium chlorate was substituted for the usual one part bone meal and two parts common salt in the salt lick. A steer partook of this lick regularly, eating about 30 Gm. each day. At the end of a fortnight the steer developed severe jaundice and became acutely ill. In all, about 285 Gm. (9½ ounces) of sodium chlorate was consumed.

Steyn,[7] in his experiments, administered 10 per cent solutions of commercial sodium chlorate by means of a stomach tube. A 5-pound rabbit tolerated 5-Gm. doses of the chemical per day on 20 successive days without ill effects, although it was killed by three consecutive daily doses of 7.5 Gm. A single dose of 10 Gm. was sufficient to kill another rabbit. Two daily doses of 15 Gm. had no visible effect upon an adult sheep while three hours after the third dose "the animal exhibited dyspnoea; accelerated heart beat which became progressively weaker; haemoglobinaemia, temperature 106° F.; visible mucous membranes, conjunctiva and unwooled parts of the skin, dirty brown in color. The animal died 20 hours after the third dose."

The administration of 30 Gm. to a sheep was followed by slight dyspnea and accelerated heart beat which passed off within a few hours. The same amount repeated on the following day produced the symptoms previously described. The maximum temperature was 108° F. and death came after 20 hours. A third sheep survived 7.5 Gm. daily for 20 days and then 10 Gm. for three days. Petechiae which lasted 24 hours appeared on the conjunctiva after the second dose and inappetence and diarrhea were noticed on the twelfth day, with complete recovery by the 17th day, although the sodium chlorate was administered daily.

Both 30-Gm. and 60-Gm. doses given to an aged horse produced a slight brownish discoloration of the conjunctiva and labored respiration, with recovery after 36 hours. A pronounced brown discoloration of the conjunctiva with ecchymoses accompanied by dyspnea followed a 90-Gm. dose, while doses of 120 and 130 Gm. produced a pronounced discoloration of the conjunctiva, somnolence, inappetence, and an accelerated and strong pulse. The temperature was 102° F., with recovery in two days.

EXPERIMENTAL PROCEDURE

Four general types of experimental work were undertaken: 1) Clinical observations and postmortem examinations of animals fed known amounts of sodium chlorate; 2) field experiments in which bindweed sprayed with known amounts of sodium chlorate constituted the sole diet; 3) methods of differential diagnosis of sodium chlorate poisoning; and 4) testing of the efficacy of various substances as antidotes for sodium chlorate poisoning.

TOXICITY OF SODIUM CHLORATE FOR CHICKENS AND SHEEP

Young chickens were fed varying amounts of sodium chlorate to determine the lethal dose, the protective action of feed, and the efficacy of the oral administration of reducing substances as antidotes. The results, shown in table I, indicate that the lethal dose of sodium chlorate for young chickens is slightly more than 5 mg. per Gm. of body weight. Those receiving food before the administration of the chemical survived slightly greater amounts, although the difference is hardly sufficient to be of importance.

Methemoglobinemia was a constant symptom in all chickens receiving sodium chlorate. The blood was abnormally dark when drawn and the clot that formed was almost black. The hemolyzed blood varied from a chocolate-brown to an almost tar-like color, due to the oxidation of the hemoglobin to methemoglobin.

Slight darkening of the blood resulted from the oral administration of 10 Gm. of

sodium chlorate crystals, while 5 Gm. produced no visible changes. Very definite darkening of the blood resulted from the administration of 30 Gm. or more, although sheep survived 30, 60, and 90 Gm. Death was produced in about seven hours by 120 Gm. The lethal dose for sheep, therefore, was found to be between 90 and 120 Gm. (3 and 4 ounces) or 2.06 and 2.5 Gm. of the chemical per kilo of body weight.

SYMPTOMATOLOGY AND GROSS PATHOLOGY OF SODIUM CHLORATE POISONING

The course of acute sodium chlorate poisoning in sheep was not marked by clinical symptoms other than somnolence and dyspnea. The temperature remained normal and hemoglobinuria was noted in but one instance. Death occurred during stupor, without convulsions. In one case, one of us (E.C.M.) was unable to determine the exact time of death in a ewe under constant observation.

Autopsy of those animals receiving lethal doses of sodium chlorate revealed flesh that was distinctly darkened, sometimes being almost black. Cut surfaces had a tendency to become lighter upon exposure to the atmosphere. The blood was black, but coagulated readily. The liver was nearly black. The lungs were the color of normal liver. The heart was dark and flabby. The spleen was dark but normal in size and the pulp was of a normal firmness.

The urinary bladder was normal, with no discoloration of the urine. The tongue, mouth, esophagus and the lining of the rumen, reticulum and omasum were free of erosions or lesions of any kind. However, in all cases there were deep, very black, eroded areas on the lining of the abomasum and the first 10 or 12 inches of the duodenum. The rest of the intestinal tract was without lesions.

SODIUM CHLORATE-SPRAYED BINDWEED PROVED NONTOXIC

The most common exposure of live stock to sodium chlorate is by the eating of sodium chlorate-sprayed forage. Tests therefore were made to ascertain whether sufficient sodium chlorate could be obtained in this way to produce clinical symptoms of poisoning. Measured areas of a heavy growth of bindweed were sprayed at the rate of 5 pounds of sodium chlorate* per square rod, the sodium chlorate being dissolved in the proportion of 1 pound in each gallon of water.

A sheep was tethered in these sprayed areas and for eleven days received no other food. The sprayed bindweeds were eaten readily. Daily bleedings revealed no discoloration of the blood, indicating that no appreciable methemoglobinemia was produced. Therefore, it can be assumed that the amount of sodium chlorate consumed by the sheep under these conditions probably was too small to produce clinical symptoms. Since our tests have shown that as little as 10 Gm. of sodium chlorate consumed at one time will produce a noticeable methemoglobinemia in a sheep, the amount of unreduced sodium chlorate remaining on the forage eaten must have been small. Inasmuch as the lethal dose of sodium chlorate for a sheep is between 90 and 120 Gm., absence of methemoglobinemia indicates a margin of safety, as far as death is concerned, of at least nine times.

DIFFERENTIAL DIAGNOSIS

The contents of the digestive system and blood serum of animals poisoned with sodium chlorate were tested for the presence of chlorates by means of ammonium thiocyanate paper, as described by Offord.[8] This method has been shown to be applicable to plant extracts and sensitive to as low as .01 mg. of chlorate per ml. of solution. In spite of this sensitivity, centrifuged fluid from the rumen of a sheep taken immediately after death failed to give a positive test. This animal had received 120 Gm. of sodium chlorate seven hours previous to death. Blood samples drawn at intervals likewise failed to give a positive result with this test. The dark color of the blood due to methemoglobin in chlorate poisoning makes the test of doubtful value, however, for blood samples.

In no case was it possible to get a positive test for chlorates upon postmortem

*Provided by the Whitman county Weed Project.

examination, indicating that the chlorate is rapidly destroyed in the animal. Chemical examination of the rumen or stomach contents therefore appears to offer little assistance to the veterinarian. Since the destruction of chlorate may give rise to both chlorine and oxygen directly, both may act as oxidizing agents in the animal.

However, differential diagnosis of acute sodium chlorate poisoning should present no unusual difficulties. The dyspnea, stupor, absence of fever with formation of large amounts of methemoglobin, giving a dark color to the blood, even where sublethal amounts of sodium chlorate have been ingested, together with the characteristic postmortem lesions and a history of possible exposure, should provide sufficient information upon which to base a diagnosis.

Care should be taken not to confuse the darkening of the blood and tissues, which frequently occurs in an early stage of postmortem decomposition. This is due to the formation of sulfemoglobin and does not show a tendency to become lighter upon exposure to the air.

The methemoglobin is most readily recognized in hemolyzed blood. It is therefore suggested that the blood samples be drawn into an equal volume of distilled water. Under field conditions tap water may be substituted. While several other compounds are capable of producing methemoglobinemia, there is little opportunity for domestic animals to come in contact with them.

REDUCING AGENTS HAVE LITTLE PRACTICAL VALUE AS ANTIDOTES

Since sodium chlorate is a strong oxidizing agent, it was hoped that reducing agents might prove to be effective antidotes, given either orally or intravenously, so as to reduce the absorbed sodium chlorate or secondary oxidizing agents before they oxidize the hemoglobin of the blood to methemoglobin.

The oral administration of a 10 per cent solution of cerelose (corn sugar), which possesses rather strong reducing power, was not appreciably effective. These results give little reason to believe that the administration of carbohydrates would prove of value in the treatment of sodium chlorate poisoning.

In the test tube sodium thiosulfate (sodium hyposulfite) readily reduces sodium chlorate. Ten per cent solutions, given orally a few minutes after the sodium chlorate, appeared to have some value in chickens, as is shown in table I. In the experiments with sheep, two hours were allowed to elapse between the administration of the sodium chlorate and the giving of the antidote. This is thought to be approximately the interval that would occur between the accidental poisoning of farm animals with sodium chlorate and the administration of the proper antidote.

Sodium thiosulfate, given either orally or intravenously; sodium galacturonate, given intravenously; and calcium gluconate, given intravenously, have no beneficial action. While the data in table I indicate beneficial results from giving sodium thiosulfate solutions within a few minutes after the sodium chlorate was administered to chickens, it is probable that this drug would have but little practical value in cases of accidental poisoning, where the interval between poisoning and treatment would be much greater. The anatomical dissimilarity of the alimentary tract of the chicken and the sheep may also be responsible for the differences observed.

The limitation of the lesions in the alimentary tract of sheep to the abomasum and upper part of the duodenum, which are known to be quite acid, led to the hypothesis that the caustic action of sodium chlorate might be prevented by alkalizing this region. However, the administration, by means of a stomach tube, of four liters of a 1.25 per cent sodium carbonate solution two hours after the sodium chlorate did not delay death nor did it materially lessen the lesions produced in the abomasum and upper portion of the duodenum. Upon autopsy, the rumen and abomasum contained copious amounts of fluid, and the rumen, abomasum and upper portion of the duodenum were alkaline to phenol red but not to thymol phthalein, which would indicate a pH between 8.4 and 9.3. The omasum

TABLE I—Toxicity of sodium chlorate for chickens.

No.	Wt. of Chickens in Grams	Preliminary Treatment	Grams $NaClO_3$ Crystals by Mouth	Mg. per Gm. Body Wt.	Antidote, by Mouth	Result
399	152	Starved 30 hrs.	1	6.58	None	Survived
396	179	Starved 24 hrs.	1	5.58	None	Died
789	186	Fed	1.5	8.06	None	Survived
332	190	Fed	1.5	7.90	None	Died 2 days
89	200	Fed	1	5.00	After 30 min. 15 cc. of 10% cerelose	Survived
398	218	Starved 30 hrs.	1	4.58	After 30 min. 15 cc. of 10% cerelose	Survived
775	230	Fed	2	8.70	After 15 min. 5 cc. of 10% cerelose	Died 7 hrs.
774	208	Starved 30 hrs.	1.5	7.21	After 15 min. 5 cc. of 10% cerelose	Died
391	156	Starved 30 hrs.	1	6.41	After 30 min. 5 cc. of 10% thiosulfate	Survived
397	208	Starved 30 hrs.	1.5	7.21	After 30 min. 5 cc. of 10% thiosulfate	Survived
27	237	Fed	2	8.4	After 30 min. 10 cc. of 10% thiosulfate	Survived
40	162	Fed	1.5	9.26	After 30 min. 5 cc. of 10% thiosulfate	Survived
399	208	Fed	2	9.61	After 15 min. 5 cc. of 10% thiosulfate	Survived
781	220	Fed	4	18.18	After 15 min. 8 cc. of 10% thiosulfate	Died 7 hrs.

presented its usual desiccated appearance and was alkaline to brom-thymol blue but not to phenol red, indicating a pH between 7.6 and 8.4.

Discussion

Methemoglobin formation has long been recognized[9] as one of the results of chlorate poisoning in certain animals. Experiments with dogs indicate that the anoxemia resulting when about 75 per cent of the hemoglobin has been converted to methemoglobin is the primary cause of death.

Blood samples drawn at intervals from the various experimental animals were analyzed spectographically for the presence of methemoglobin. The smallest dose showing any spectrum lines of methemoglobin was 10 gm., or approximately one-tenth the lethal dose. Since about 25 per cent of methemoglobin must be present in order to be visible in the spectroscope (Kobert[10]), it is probable that even smaller amounts would cause methemoglobin formation. It must be remembered also that these amounts refer to single dosages and not to continued ingestion of chlorate, as might be the case where the animals consume chlorate-treated forage.

The characteristic lesions in the abomasum and duodenum were observed in all lethal cases of chlorate poisoning, but the extent of their formation following sublethal doses and continued doses is unknown. Likewise, whether or not the formation of the lesions precedes the formation of methemoglobin or whether methemoglobin may be formed from dosages too small to cause erosion of the digestive tract, is at present undetermined.

Following heavy doses methemoglobin was noticed as early as two hours, while not for five to seven hours in smaller doses. In a sheep surviving 90 Gm., methemoglobin had disappeared from the blood spectroscopically in 72 hours. How long the erosion of the abomasum, if any, persisted is of course not known. These questions will be the subject of a further study. Because of these uncertainties it is not possible to state at present to what extent animals can be permitted to consume sodium chlorate-treated forage without being harmed physiologically. On the other

hand, if the recommended amounts and procedures are used for weed eradication, it is doubtful if the resulting forage would cause death from sodium chlorate poisoning.

The use of sodium chlorate in eradicating noxious weeds has interest to the veterinary profession other than its direct toxicity to stock, as it appears to offer a most practical way of exterminating poisonous plants on some ranges and farms.

SODIUM CHLORATE POISONING IN WASHINGTON

Case 1.—Near the end of August, 1937, the sudden deaths of range cattle in central Washington were investigated.

One very sick cow had returned home and aborted. Breathing was labored and she refused to eat, although she drank water freely. Her temperature was 104.5° F. Her eyes were sunken, her feces dark and fluid, and she showed incoördination and walked with difficulty. The urine was scant but clear. Autopsy of the last steer found dead revealed a carcass in which the flesh was almost black and the blood tarlike and coagulated. The spleen was of normal size and consistency. The only lesions in the alimentary tract consisted of deep, black, eroded areas on the walls of the abomasum and smaller areas of erosion on the first 10 or 12 inches of the duodenum. Postmortem decomposition prevented more detailed examination.

Investigation revealed that sodium chlorate had been applied by relief labor to control noxious weeds. Examination of the treated areas in the valley showed that the crystals had been lightly broadcast at the rate of 4 pounds per square rod. Cattle and horses had remained in some of the pastures containing treated areas and showed no symptoms. However, further up in the mountain ranges a number of dead cattle were reported. Five were scattered over a small meadow below a deserted cabin. Two had aborted, although in their state of decomposition it was impossible to ascertain whether this had occurred before death.

Small areas, at one time tilled, had grown up with Canada thistle. Drums containing 50 pounds of sodium chlorate had been taken to these areas and, in this particular place, apparently an entire drum had been used, although only a few square rods contained thistles. The crystals tend to form lumps in the drums and these had not been broken up, so that a number of lumps the size of walnuts still could be picked up. Manure and tracks showed that cattle had spent considerable time in these treated areas. Because of the danger of fire in raking up sodium chlorate which has partially acted upon organic matter, the treated areas were immediately fenced and the sodium chlorate crystals dissolved and washed into the ground with water. Following this, no more losses occurred.

It was recommended that the use of sodium chlorate in the control of noxious weeds be continued, but that the following precautions be taken:

1) The range be heavily salted at least a week before the sodium chlorate is applied, and the salt be replenished frequently so that plenty will remain in the troughs as long as there is any sodium chlorate on the ground;

2) all lumps of sodium chloride either be broken up or dissolved in water;

3) that only reasonable amounts, *i. e.*, not over 5 pounds per square rod, be applied;

4) that great care be taken not to spill the chemicals on the ground where cattle would have access to small piles; and

5) that partially used drums be placed where cattle can not reach them.

Following this, both cattle and sheep have died following access to sodium chlorate-sprayed areas.

Case 2.—Sodium chlorate was used to spray an area of about 10 square rods of Canada thistle scattered along a logging railroad running through a low, marshy pasture.

The plants found on the pasture were:

Red top (*Agrostis alba*).

Timothy (*Phleum pratense*); in dried portions of the pasture.

Sedge (*Carex arenaria*); predominates.

Dandelion (*Taraxacum officinale*); scattered throughout.

Aster; trace.
Rush (*Juncus tenuis*); trace.
Plantain (*Alisma Plantago*); trace.
Bull thistle (*Cirsium lanceolatum*); trace.
Orchard grass (*Scolectotrichum graminis*); trace.
Kentucky bluegrass; trace.

Willow and cottonwood trees were also present. Not all of the pastured land was examined, but on the portion observed no water hemlock was found. The men who did the spraying reported that the cattle grazing in the pasture at the time of spraying had been salted about a day previous. No salt was observed upon examination of the area several days later.

Two cows were dead the morning following the application of the spray and a third cow died a few hours later. Methemoglobin was found in samples of blood sent to the state college.

Examination of the pasture revealed that the sprayed areas had been grazed heavily, although ample forage remained in the rest of the pasture. The tips and buds of the sprayed thistles were eaten off and the sedge and grass were eaten off to within a few inches of the ground. On one of the larger sprayed patches of about 4 square rods, it was estimated that approximately 250 pounds of the treated forage had been consumed.

It was obvious that the cattle liked the chlorate-sprayed weeds very much, even though they may have been well salted. The intense grazing on the sprayed area may indicate increased palatability of the forage as a result of the chlorate or a mere consummation of the forage in an effort to get the chlorate. Quite probably, they should have been salted heavily for some time previous to the spray rather than immediately in advance of it.

Case 3.—Canada thistles on the banks of a ditch running through a pasture and in a yard cut off from the pasture by a fence were sprayed with sodium chlorate. Two weeks later, sheep were placed in the pasture. Although the attendant stated that the sheep had been well salted and that both blocks of mineralized salt and coarse ground salt were observed on the ground when the area was examined, the sheep congregated in the sprayed areas first, eating all of the sprayed vegetation. Several of the sheep broke through the fence into the yard and avidly ate the sprayed vegetation. At the time of observation the sprayed areas were almost bare, only the short stumps of the thistles remaining. The ground itself appeared to have been disturbed, as if it had been licked by the animals.

Several sheep died in the fenced yard as well as a number in the main pasture. The sick sheep were observed to drop behind the rest of the flock and could not be made to move rapidly. Some lived two or three days. In addition to the dark blood and flesh, which is typical of experimentally produced sodium chlorate poisoning, these sheep showed intense inflammatory changes in the lining of the rumen.

SUMMARY

The lethal oral dose of sodium chlorate for young chickens is slightly greater than 5 mg. per Gm. of body weight; for sheep it is between 2.06 and 2.50 mg. per Gm. of body weight. Thus, sheep weighing between 90 and 100 pounds survive 90 Gm. but succumb following the administration of 120 Gm.

Somnolence and dyspnea are constant symptoms and doses as small as 10 Gm. produce detectable amounts of methemoglobin in the blood of sheep.

Marked darkening of the blood, muscles and viscera due to the presence of methemoglobin, together with deep, black erosions in the lining of the abomasum and upper part of the duodenum, are the characteristic pathological changes found in acute fatal cases. Neither the administration of reducing agents nor the alkalization of the digestive tract with copious amounts of sodium carbonate solution, two hours after the sodium chlorate, prolonged the life of sheep fed sodium chlorate. No successful antidote for sodium chlorate poisoning in sheep has been found.

Bindweed, sprayed with 5 pounds of sodium chlorate per square rod, did not prove toxic to a sheep fed no other food over a period of eleven days.

It is possible that sodium chlorate-sprayed Canada thistle is toxic. Whether this is due to the formation of some new substance or to the fact that the sprayed thistle is eaten readily is not known.

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Trichomoniasis of Cattle

As a cause of sterility in cattle, trichomoniasis was described vaguely in 1928. Since then, this protozoan infection has been given a place of importance among the causes of interrupted reproduction. The work and observations of Cameron, Fincher and Gilman, of McNutt, Walsh and Murray, of Lothe, of Hess and others in all parts of the world support the theories evolved from the studies of this newly identified infection of the genital tract. In deliberations over sterility problems *Trichomonas fetus* is no longer overlooked.

Plow Deeper

FOOD AND FEED contain certain rare elements in minute quantities that are needed to maintain the normal processes of living things. But, inasmuch as a given amount of the crust of the earth where food and feed grow contains but a given amount of these elements and Nature provides no means of redistribution, every particle of vegetation carted away from the land where it grows deprives the soil of a definite quantity of them. To contradict this is not good arithmetic.

The arable soil becomes progressively depleted as soon as it is used for farming purposes, and the sum of the depletion is expressed in the form of the deficiency diseases that are now baffling the student of scientific medicine. Though some capable authorities tend to discount this phase of etiology, the number of them is decreasing. Boiled down to practical terms, food and feed are not as good as they used to be. The milligrams of cobalt, zinc, iodine, manganese and other lesser minerals carted away on the hayrack or in the bodies of market animals are not prone to be redistributed over the land. Little of them ever comes back. Nature seems to have risked everything upon the minute amounts of these essential elements animals and plants require. Obviously, Nature's intention was to move its inhabitants to new pastures when soil privation had become precarious.

G. D. Jones, writing in *Agricultural Engineering*, says that the remedy for soil depleted of needed elements is plowing to greater depths. A new plow, called "the ripper," can dig to the depth of 3 feet.

Most all of the food we eat contains a large percentage of water. Good, lean beef, for example, contains 69 per cent of moisture (water).

Including loss of time, drugs, doctor bills, nursing, hospitalization, etc., sickness costs the American people 10 billion dollars a year.

Results of Blood Cultures on Seven Heifers Artificially Infected with Brucella Abortus*

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IN AN EARLIER paper[1] we reported the work done on two of the animals included in this paper, Nos. 342 and 349 (see table II). A discussion of blood-culture methods can also be found there. The method of Soule,[2] who reported a study on a large series of animals, was not found satisfactory, as many contaminations occurred in cultures when this method was used. Soule incubated 50 cc. (1.7 oz.) of whole blood in 450 cc. (15 oz.) of glycerol infusion broth under 10 per cent carbon dioxide and made blood cultures on more than 5,000 ordinary "herd-run" cattle. Two series of tests were made on each animal at intervals of approximately six months. In the first series of tests, agglutinins were present in the blood of 2,237 of the animals but only 299 gave positive blood cultures. In the second series agglutinins were present in 2,607 cases, with 206 positive blood cultures. There were 40 positive blood cultures without concomitant agglutinins.

Lübke[3] found a bacteremia in 90.4 per cent of 21 recently infected animals and in 5.9 per cent of 81 cases of old infection. His studies were made by inoculating guinea pigs with 5 cc. (75 mm.) of citrated blood and later culturing the guinea pigs.

Haring, in a personal communication to us, reported the results of blood cultures on cattle after infection by way of the conjunctiva. Three animals were inoculated on October 26. Blood cultures were made October 27 and 30, November 2, 7, 16, 23, and 30; December 1, 4, 7, 14, 21, and 28; January 4, 11, and 29; February 15; and March 28. *Brucella abortus* was isolated from all three animals on November 7 and from one of the three it was isolated on November 16. The first isolations in each case were made twelve days after inoculation. Two other animals were exposed to infection November 30 and *Br. abortus* was isolated December 7 in each case, or seven days after exposure. It also was isolated from the blood of one animal on December 21. Cultures were made November 30; December 1, 4, 7, 14, 21, and 28; January 4, 11, and 29; February 15; and March 28.

Cotton[4] reported the collecting of blood from the jugular vein of 13 actively infected cows in different stages of pregnancy and injecting 3-cc. (45-mm.) doses into guinea pigs. The pigs were killed two months later and examined for lesions. The spleens were cultured for *Br. abortus* and the blood was tested for agglutinins. No evidence of *Br. abortus* was found in any of the guinea pigs. In the same paper he reported that Buck, one of his associates, had succeeded in isolating *Br. abortus* from the blood stream of two pregnant cows 52 and 62 days after intravenous injection, employing a technic described by Shaw.[5]

Since our first paper further work has been done to compare methods of blood cultures. We reported from comparative studies made on guinea pig blood that the heated citrate method recommended to us by Haring[6] and the use of liquoide "Roche"[7] were the most efficient methods. It was decided to use these two methods as well as inoculation of guinea pigs with 5 cc. (75 mm.) of whole blood for further comparative studies. One hundred and twenty-three comparative tests were made with these three methods, the blood of two cows (383 and 384B) being used.

Br. abortus was isolated 16 times by the heated citrate method, six times by the use of liquoide and six times by the guinea

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pig-inoculation tests. *Br. abortus* was isolated 15 times by the heated citrate method on days when it was not isolated by any other method, four times by the use of liquoide when it was not isolated by any other method, and five times by guinea pig inoculation when it was not isolated by any other method. The organism was isolated on one day by both the guinea pig-inoculation and the liquoide methods, and on one day by the heated citrate method and the liquoide.

TABLE I—Results of the studies of comparative methods.

Heated Citrate	Liquoide	Guinea Pig Inoculation
15	—	—
—	4	—
—	—	5
—	1	1
1	1	—

On many occasions only one or two colonies of *Br. abortus* were found on the heated citrate cultures. This would indicate that the number of organisms per cc. of blood was small and probably not sufficiently numerous to infect guinea pigs.

The liquoide method involves the collecting of 10 cc. (150 mm.) of blood into 2 cc. (30 mm.) of 1 per cent aqueous solution of liquoide and incubation of the entire amount in one container. If the cultures made on liquoide were contaminated, subcultures were made on gentian violet plates (1-200,000 gentian violet), and one guinea pig was inoculated with the culture. This examination was not considered negative for Brucella until the guinea pig was autopsied and cultured four weeks later. Two of the isolations made by the use of liquoide were carried out in this manner.

Further comparative studies of different methods of blood cultures also were made with the blood of guinea pigs. In these experiments the use of liquoide seemed to have a decided advantage. Guinea pigs were inoculated with *Br. abortus* and cultures were made from the heart blood 48 hours after inoculation. The hair was clipped from the cardiac area and the skin disinfected with iodine. Cardiac punctures were made and 6 cc. (90 mm.) of blood was withdrawn. This was used in 1-cc. (15-mm.) amounts to compare six different technics. One cc. of the blood was allowed to clot and the serum was removed. The clot was then placed in a tube of infusion broth and incubated. This is a method of blood culture described by Rainsford.[8]

The technic of Boez and Robin[9] also was used. In this method the blood was mixed with an acid citrate, which prevented its clotting and reduced the pH to approximately 5.5, thus destroying its bactericidal action. The acidity was counteracted by transferring the acid mixture to an alkaline broth pH 8.3 for incubation. The mixed blood and culture medium attain a pH of approximately 7.5, which is suitable for growth. Heated citrate and liquoide methods also were used as well as cultivation of 1 cc. of whole blood in simple infusion broth. The effect of Victoria blue[10] added in 2 per cent quantities to infusion broth also was studied. Fifty-nine pigs were cultured, twelve of which gave entirely negative results by all methods. Liquoide gave 37 positive cultures, acid citrate 30, heated citrate 27, whole blood 27, clot 15, and Victoria blue 15. Stewart *et al*[11] also found that liquoide gave better results than other methods tested by them.

The liquoide or acid-citrate methods were not adopted for routine blood cultures on cattle, however, because they involve the incubating of the whole amount of blood taken in a single container. Thus, if any contaminating organisms were present, the culture was quickly overgrown with such organisms. These methods present the same difficulty as the method used by Soule —that of contamination. In our experience it was very difficult to free the skin of the cow of bacteria in order that sterile blood cultures could be obtained. However, this difficulty was not encountered when blood cultures were made on guinea pigs and horses. Blood drawn from horses, with the same aseptic precautions as used with cows, rarely became contaminated. Soule did not state how he prepared the skin of the animals studied. We used several different methods, none of which was found entirely satisfactory. Blood cultures were made daily and the use of iodine for

disinfecting the skin was found to be irritating and appeared to show little advantage over the method finally adopted.

EXPERIMENTAL PROCEDURE

A study was made of seven heifers artificially exposed to *Br. abortus*. All animals were calves 9-10 months of age obtained from the local stock yards and brought to University Farm, where they were held in isolation for a period of five to six months and bred before being exposed to infection. All animals gave negative reactions to the agglutination test for Bang's disease during this period (1:25 dilution). Exposure was made by drenching with physiological saline suspensions of placentas or stomach contents of aborted fetuses from which *Br. abortus* had been isolated, or by dropping a saline suspension of the organism into the conjunctival sac. Single exposures to infection were made at different stages of pregnancy (60, 70, 118, 127, 129, 130, and 159 days in gestation). Each animal was held in a separate box stall where every precaution was taken to avoid infection from sources other than the known exposure.

Daily blood cultures and tests for agglutinins of *Br. abortus* were made after exposure. When the agglutinins of the blood reached a dilution of 1:100, the animal was considered positive. The three animals infected by way of the conjunctiva became positive 9, 10, and 29 days later, or an average of 16 days after contact with Brucella. Three heifers that were drenched became positive 11, 27, and 74 days later, or an average of 37 days after exposure, and one animal, though giving a slight antibody response, never reached the positive range. The studies on this small series of animals would indicate that infection takes place more readily *via* the conjunctiva than by drenching.

The blood-culture method finally adopted was that used by Haring. This technic, when compared with several methods, gave us the best results for obtaining blood cultures on cattle. Only a small quantity of blood was cultured (10 cc.), and it is possible that on occasions Brucella was not isolated, although it was present in the blood. Cow 383 (see table II) at one stage in her infection yielded the organism regularly on culture. This probably was due to the fact that there were present suppurative lesions that served as a reservoir and from which Brucella was isolated.

The blood cultures in this experiment were made in the following manner. The hair was clipped and shaved from the neck of the animal in a wide area along and around the jugular furrow. The entire neck was then washed with soap and warm water and a stiff hand brush was used to scrub the skin of the clipped area. The soap was removed by rinsing with clear, warm water. The area directly over the jugular vein was swabbed with a 1:500 alcoholic bichloride solution.

Ten cc. of blood was drawn directly into sufficient sterile citrate to prevent clotting. The apparatus for collecting blood was prepared in advance and autoclaved 15 pounds for 20 minutes. This apparatus consisted of a glass test tube containing citrate into which a piece of rubber tubing was inserted within a cotton plug. One end of the rubber tubing was allowed to hang free inside the citrate tube; the other end was slipped over one end of a piece of glass tubing 2 inches long; another piece of rubber tubing was placed on the free end of the glass tubing. A vena puncture needle was attached to the free end of the rubber tubing. A small test tube was used to cover the needle and was held in place by a cotton plug. The rubber tubing was large enough to fit tightly on the shank end of the bleeding needle.

Every aseptic precaution was taken at the time the blood was drawn. The vena puncture needle was not exposed to the air until the skin of the cow was prepared for bleeding. A vena puncture was made. When sufficient blood flowed into the citrate tube, a pinch clamp was placed on the rubber tubing leading from the needle, the rubber tubing was removed from the glass tubing, thus leaving the vena puncture needle still in the jugular, and additional blood for agglutination tests was collected before the needle was withdrawn.

The blood taken for culture was mixed

with the citrate by shaking, and the mixture was placed in a water bath at 57° C. for 15 minutes. After heating, the blood was withdrawn from the tube by a 10-cc. pipette and distributed over approximately 20 serum-agar slants. These were incubated under 10 per cent carbon dioxide and examined twice a week thereafter. The examination consisted of observing the surface of the slant for colonies of *Br. abortus*. If none were present, the blood at the bottom of the slant was tipped over the surface and the tubes were put back to incubate.

Incubation and examinations were made over a period of approximately one month. Some difficulty was experienced with contamination by moulds on the culture tubes during the third and fourth weeks. All such tubes were discarded. Isolations of *Br. abortus* were made well within the first two weeks of incubation and, in our opinion, the one-month period of incubation gave no added advantage. Many times only one or two colonies were found on one agar slant of the 20 inoculated. These colonies were subcultured. Identification was made by the usual biochemical tests and also by making antigen of the cultures, and using it in tests with known positive and negative abortus serums.

We have stated that the skin of the cow is difficult to free sufficiently of bacteria in order to obtain uncontaminated cultures when quantities of blood are drawn directly into broth. Usually, a small piece of the skin was washed into the blood used for culture when the needle was forced through the skin. It was no doubt this small piece of skin which carried the bacteria into the cultures. By the method adopted, if a few bacteria from the skin escape into the blood during the process of collection, all cultures are not contaminated, as the blood is distributed into 20 different cultures and, if contamination has occurred, only one or two agar slants are affected. The heating process at 57° C. is claimed by the originators of the method to destroy bactericidal substances in the blood so that the growth of Brucella will not be inhibited. Another advantage of the method is the easy demonstration of Brucella on the cultures.

RESULTS

The period of time after exposure to the first isolation from the blood stream varied from 6 days to 7, 11, 21, 29, 60, and 71 days. The average time before the organism was isolated in the blood stream, including both methods of exposure, was 29 days. There appeared to be little difference in the method of exposure and the appearance of the organism in the blood stream. In all cases, with the exception of one, Brucella was isolated from the blood shortly after or before the blood titre reached 1:100. In this particular case, 384B, *Br. abortus* was not isolated from the blood stream until 42 days after the blood titre reached 1:100. This animal was infected through the conjunctiva with a strain of Brucella isolated from a moose. The strain possessed all of the cultural characteristics of an abortus strain, but did not in this case produce an abortion. The pregnancy terminated at 260 days, and *Br. abortus* was isolated from the colostrum and placenta but not from the calf, which was destroyed and cultured six days later. Cultures and guinea pig inoculations were made of a large number of lymph nodes and various other tissues.

The number of times *Br. abortus* was found in the blood stream varied from a single isolation from cow 453, which did not become positive to the agglutination test, to 22 isolations, extending over a period of 114 days from animal 383. An isolation was made from her on the day she was slaughtered. These two animals represent the extremes of infection. Cow 453 gave only a slight antibody response, and on August 5, 17 days after exposure, she gave a 1:50 agglutination reaction. This titre gradually diminished until, on November 5, she failed to react in a 1:5 dilution. Isolation of *Br. abortus* was made from the blood stream on the 21st day. This indicated a definite infection with Brucella but one which was evidently cast off quickly, as the organism could not be isolated from the placenta or colostrum at the time of

TABLE II—Artificial infection of seven heifers with Bang's disease.

Cow No.	Method of Infection	Agglutination Reaction at 1:100	First Isolation of Br. Abortus from Blood Stream	Length of Time Br. Abortus Was Isolated from Blood Stream; Dates Isolated	Remarks
342	Drenched with 350 cc. of saline suspension of Brucella-infected placenta. Jan. 28, 1936 (129 days in gestation)	Feb. 24, 1936 (27 days after exposure)	Feb. 26. 29 days after exposure	82 days. 2-26, 3-2, 3-7, 3-11, 3-18, 3-24, 3-27, 5-1, 5-19	Aborted May 18, 1936, at 239 days. *Br. abortus* isolated from colostrum, placenta and fetus. Experiment terminated July 7, 1936
349	0.5 cc. of saline suspension of *Br. abortus* in conjunctival sac. Feb. 13, 1936 (127 days in gestation)	Incomplete 1:100 Feb. 23, 1936 (10 days after exposure)	Feb. 24. 11 days after exposure	70 days. 2-24, 3-3, 3-4, 3-11, 3-28, 5-1, 5-5	Aborted May 11, 1936, at 214 days. *Br. abortus* isolated from colostrum, placenta and fetus. Experiment terminated July 7, 1936
383	Drenched with 500 cc. of stomach contents Brucella-infected fetus Feb. 11, 1937 (118 days in gestation)	Complete reaction 1:100 April 26, 1937 (74 days after exposure)	April 12. 60 days after exposure	114 days. 4-12, 6-12, 6-26, 6-29, 7-1, 7-2, 7-3, 7-9, 7-10, 7-12, 7-14, 7-19, 7-20, 7-21, 7-24, 7-26, 7-27, 7-28, 7-30, 7-31, 8-4, 8-5	Dead fetus removed 211th day of pregnancy, May 15, 1937. Placenta retained. *Br. abortus* was isolated from colostrum but not from fetus or placenta. Experiment terminated Aug. 5, 1938
384B	1.5 cc. saline suspension *Br. abortus* culture isolated from moose placed in conjunctival sac. April 1, 1937 (130 days in gestation)	Incomplete reaction 1:100 April 29, 1937 (29 days after exposure)	June 10. 71 days after exposure	106 days. 6-10, 6-24, 6-25, 7-7, 7-12, 7-31, 8-20, 9-16, 9-17, 9-20, 9-21, 9-23, 9-24	Calved Aug. 8, 1937, at 260 days. *Br. abortus* isolated from colostrum and placenta. Not isolated from calf. Experiment terminated Oct. 5, 1937
454	Three drops of saline suspension *Br. abortus* in conjunctival sac. July 12, 1938 (159 days in gestation)	Incomplete 1:100 July 21 (9 days after exposure	July 18, 1938. 6 days after exposure	9 days. 7-18, 7-19, 7-20, 7-21, 7-26	Aborted Aug. 29, 1938, at 207 days. *Br. abortus* isolated from colostrum, fetus and placenta. Experiment terminated Nov. 19, 1938. Br. abortus isolated from milk Sept. 23, and Nov. 19, 1938
452	Drenched with stomach contents Brucella-infected fetus. July 19, 1938 (70 days in gestation)	Incomplete 1:100 July 30, 1938 (11 days after exposure)	July 26, 1938. 7 days after exposure	39 days. 7-26, 8-7, 8-9, 8-10, 8-11, 8-17, 8-19, 8-20, 8-29, 9-1, 9-2, 9-3, 9-6	Aborted Sept. 19, 1938, at 132 days. *Br. abortus* isolated from colostrum, placenta and fetus. Experiment terminated Nov. 11, 1938
453	Fed stomach contents Brucella-infected fetus (same material as 452). July 19, 1938 (60 days in gestation)	Highest titre 1:50 Aug. 5, 1938	Aug. 9, 1938. 21 days after exposure	8-9	Calved Feb. 20, 1939, 276 days. Cultures of the calf, colostrum, milk and placenta were negative for *Br. abortus*

calving or from milk samples examined during the lactation which followed.

Animal 383 was also an interesting subject, since her agglutination titre did not reach the positive range until 74 days after exposure. It was necessary to remove a dead fetus on the 211th day of gestation. The placenta was retained. *Br. abortus* was not isolated from the fetus but it was isolated from the placenta, colostrum, and milk. Shortly following parturition a large abscess was located on the ventral surface of the abdomen. Starting approximately a month after parturition, isolations of *Br. abortus* were made almost daily until the animal was destroyed. Cultures made on tissues obtained at autopsy yielded Brucella from the mesenteric, deep inguinal, iliac, and supramammary lymph glands. The organism also was found in the udder, the spleen, and in the abscess on the abdominal wall. *Corynebacterium pyogenes* also was isolated from the abscess.

This animal was interesting because of the long incubation period, 74 days, and also because of the severe generalized infection. The presence of *C. pyogenes* in the abscess as well as Brucella indicates that the suppurative effect might have been produced by the former, as it is commonly found in such lesions. The animal is a classic example of the manner in which sterility may be produced in Bang-infected animals. Placentitis was pronounced and later aggravated by trauma produced in the attempt to secure fresh placenta for bacteriological study. Secondary invading organisms produced suppurative lesions of such nature and extent that the patient was rendered hopelessly sterile.

Br. abortus was isolated from animal 384B over a period of 106 days. The last isolation was made eleven days before slaughter. This bacteremic period probably would have persisted had she been studied longer. Isolations of Brucella from the blood stream in the three-week postparturient period equaled those obtained in the five-month period preceding parturition. Since this animal gave birth to a living calf, she probably possessed a certain amount of resistance.

The last isolation from animal 349 was made 63 days before slaughter. Only eight isolations were made from the blood stream, all before the termination of pregnancy.

The last isolation from cow 342 was made 47 days before slaughter. One isolation was made the day after calving. All other isolations were made before the termination of pregnancy.

Thirteen isolations of *Br. abortus* were made before animal 452 aborted; none was made afterwards. This animal was removed from the experiment 65 days after the last isolation.

The first isolation of *Br. abortus* was made from animal 454 six days after exposure to infection and was isolated only five times over a nine-day period. All isolations were made before the abortion occurred.

DISCUSSION

A definite bacteremic period shortly after exposure and at the time agglutinins were beginning to appear in the blood stream is seen in a study of table I. Animal 384B was the only one of the seven animals studied in which a bacteremia was not demonstrated at this time. In animal 453, although she never became definitely positive, *Br. abortus* was isolated 21 days after exposure to infection and near the time the agglutinins reached their highest point, which was 1:50. Bacteremia may continue until the time of calving or abortion. In three animals—349, 452, and 454—*Br. abortus* was isolated before paturition but not afterwards. *Br. abortus* was isolated eight times from cow 342 before she aborted and once on the day after. No isolations were made later, although cultures were continued for approximately 1½ months. In two animals, 384B and 383, *Br. abortus* was isolated before and after parturition took place. Animal 384B gave birth to a living calf and Brucella was isolated six times before parturition and six times after parturition. The last isolation from the blood stream was made eleven days before the experiment was ended. Animal 383 might be considered an example of a persistent bacteremia which occurred

after a suppurative localization of Brucella. Animal 453 appeared to be resistant, and even though *Br. abortus* was found in the blood stream on one occasion 21 days after exposure, she never developed a positive agglutination titre. *Br. abortus* was isolated from cow 454 only over a nine-day period and, although organisms were being excreted from the udder for a period of approximately three months after the abortion occurred, the organism was not demonstrated in the blood stream during this period.

This experiment shows that *Br. abortus* can be isolated from the blood stream of Bang-infected cattle during the incubation period and until the time of parturition. In two animals it was demonstrated after parturition. It would be necessary to study animals over several lactation periods in order to learn the incidence of *Br. abortus* in the blood stream. This, unfortunately, we have been unable to do, because of lack of buildings and ground.

Two rather interesting points were brought out in the study. First, the differences in resistance to infection are well demonstrated. Cow 453 received the same treatment as cow 452, yet agglutinins for *Br. abortus* in animal 452 reached 1:100 in eleven days and cow 453 never attained an agglutination titre higher than 1:50.

The second point of interest brought out in this study is the variable incubation period. By this is meant the period of time from exposure until the agglutination titre of the blood for Brucella reached 1:100. The shortest period was nine days and the longest, 74 days. It is evident that a long incubation period, such as found in animal 383, presents a difficult problem to those interested in the control of the disease by periodic blood testing. If the second test had been made 60 days after the first test, this animal still would not have been considered positive. The titre of this animal went up very gradually from an incomplete reaction at 1:10, which she had at the time of infection (February 11), to 1:25 on March 27. An incomplete reaction at 1:50 was reached on April 4, when she would have been considered a suspect. This was 52 days after exposure. Animals such as this are no doubt the cause of certain herds' being difficult to free of the disease by the blood-testing method.

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Brucella Abortus

Great disputes have arisen over the part played in abortion by various microörganisms since Professor Bang discovered *Brucella abortus* in 1897 because all observers could not reconcile their experiences with the exclusive specificity claimed for that microbe. Obviously, however, with that microbe out of the picture, the breeding troubles of the dairy industry will be manifestly reduced and, besides, none of the other pathogens associated with sterility and abortion endanger human health.

Certified Milk

If certified milk is to remain the "honor milk" of the United States, it then deserves the highest type of control available. The center of control should be a medical milk commission, not one in name alone, but comprised of physicians who are actually interested in the production and consumption of high grade milk. Control should be directed through the sanitarian, whose qualifications should include a vast knowledge of milk hygiene, veterinary medicine, public health, and good common sense.—*From Certified Milk, September 1939.*

A Chemical Study of Ketosis in a Dairy Herd*

By C. W. DUNCAN,† C. F. HUFFMAN,‡ and H. A. TOBIN,§ D.V.M.

THE EXPERIMENTAL work reported in this paper was originally initiated for the purpose of alleviating and studying acetonemia in a herd of purebred dairy cattle under farm conditions. The disease became so malignant that all of the milking cows, first-calf heifers, and open heifers were becoming emaciated and one cow had died. A survey of the literature on acetonemia, or ketosis, in dairy cows reveals that this disease has been recognized as a distinct entity for only a relatively short length of time. Parturient paresis, post-parturient dyspepsia, atypical milk fever, or after-calving indigestion, however, have been long recognized by the veterinarian as a typical condition associated with the first six to eight weeks following parturition. Stinson[30] was among the first to draw attention to the fact that these conditions were constantly associated with a well-marked ketosis.

All of the available data on the concentration of the total ketone bodies in the blood and urine of normal or apparently normal dairy cows have been summarized in table I. From an examination of this table it may be concluded that the total ketone bodies in the blood of the normal pregnant cow or of the cow that has recently freshened may reach a maximum value of 5 or 6 mg. (expressed as acetone per 100 cc. of whole blood) without evidence of any abnormal physiological manifestations. The mean value for the recorded data is 2.99 mg., with variations from traces to 5.54 mg. Likewise, it may also be concluded that the total ketone bodies in the urine may reach a maximum value of 15 or 20 mg. without evidencing abnormal manifestations. Koffman,[14] however, believes that amounts of ketone bodies in the urine less than 6 to 8 mg. are normal but that ketonuria is indicated when the amount of ketone bodies exceeds these figures. The maximum values reported by Sjollema and van der Zande[33] and Little and Wright[15] are considerably out of line with those recorded by the other investigators. The mean urinary value for these data is 10 mg. Carpenter's[5] results with fasting steers indicate that these animals do not exhibit a tendency toward ketosis during fasting, which suggests the possibility that the ratio of carbohydrate to fat is not necessarily the cause of ketosis but that it may be due to some other factor.

Table II summarizes all of the available data on ketone bodies in the blood and urine and the calcium, inorganic phosphorus, and carbon dioxide content of the blood plasma of cows suffering from acetonemia or acetonemia with complications.

From a review of the available literature, it appears that the term "acetonemia" has been used rather loosely in speaking of this disturbance. The term "ketosis," which implies the abnormal accumulation of the ketone bodies (acetone, acetoacetic acid and β-hydroxybutyric acid) in the blood (ketonemia) and the excessive excretion in the urine (ketonuria) is, from a scientific standpoint, a more specific designation of the condition because the abnormal concentration of ketone bodies is not confined to the blood alone but also to the urine and milk.

Since this disease became recognized, its economic importance from the standpoint of loss in flesh, milk production, and production of off-flavored milk[1] has become more apparent. The results of this experiment are of considerable practical importance to the veterinarian not only because of the fragmentary knowledge of the cause of the metabolic failure in the cow but also in supplying additional factual information

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on the blood chemistry of the dairy cow under these conditions.

EXPERIMENTAL PROCEDURE

The herd of cattle studied in this report consisted of nine mature, purebred Jersey cows, three first-calf heifers, and three open heifers. The ration which the animals had received during the fall and winter months consisted of poor quality soybean hay, corn silage, and a mixture of grains (approximately 3 parts of ground speltz and 1 part of ground corn).

The occurrence of the disturbance in the health and milk production of the cows became most pronounced within two to six weeks following a normal parturition. It was the owner's opinion that the cows were in good nutritional condition before calving and that all had begun to produce large quantities of milk. He also observed that the cows lost appetite gradually, rapidly decreased in milk production, became constipated, hide-bound, listless, and emaciated in appearance. He reported that the barn had a peculiar odor at times. The case was immediately diagnosed by his veterinarian as the nonparturient digestive type of acetonemia, the syndrome of which has been outlined by Fincher.[10]

The treatment, in general, consisted of the immediate administration of chloral hydrate (1 dram in 12 ounces of warm water, three times per day for one day) to the cows in the most serious condition. The soybean hay was replaced at once by 10 pounds of medium-quality alfalfa hay per day for each cow plus all the corn stalks and all the additional alfalfa hay that the cows would consume. Silage feeding was discontinued for the first two weeks. During the first week, the severely affected cows were given all the corn sugar that they would eat once a day (usually 1 to 2 pounds) and then all the warm water that they would drink. By the end of the week, the corn sugar was replaced by molasses and each of the animals received 2 pounds of molasses per day. In addition to the above feeding schedule, each of the cows received the grain mixture at the rate of 5 pounds per day for the first two weeks. The changed feeding schedule produced noticeable improvements in the majority of them within two weeks but, due to the condition of some of the cows, the disease was not rapidly amenable to treatment.

The chloral hydrate was first administered on February 6, 1938, at which time a few blood samples were obtained for chemical analysis. The feeding schedule was initiated on February 8, and at that time blood and milk samples were secured. Blood and urine samples were taken again on February 11, March 5, April 8, and urine samples on April 29. Owing to the distance of this herd from the college laboratory, it was not feasible to secure samples at more frequent intervals in order to follow the blood and urinary changes in detail. Because of the emaciated condition of some of the cows and the slow response of the others to the changed feeding practice, it was necessary to administer chloral hydrate to all of the milking cows on February 17. On March 3, the drug was again administered to cows 94728, 488602 and 488608.

By March 10, the feeding schedule had completely reverted to the soybean hay, corn silage and small portions of the grain mixture. The cows continued on this ration until they were turned out to pasture on April 28.

During the course of this investigation, qualitative tests for the presence of acetone bodies in the urine of the cows were made in an attempt to correlate the degree or intensity of the color produced with the actual quantity of ketone bodies found by the quantitative procedure. Therefore, a systematic examination of the urine was made in the barn at each visit by the ammoniacal sodium-nitroprusside test and the degree of intensity or absence of the permanganate color was recorded. The efficiency of this test is known to depend upon the presence of small amounts of acetoacetic acid or acetone but the test often failed to indicate a positive color reaction, whereas the quantitative test by the Van Slyke[24a] procedure frequently gave the opposite result. The qualitative test does not give evidence of the presence of β-hydroxy-

butyric acid, although quantitatively this acid is reported to be the most important one of the keto acids.[3, 5]

In general, it was found that when the concentration of the total ketone bodies was less than 25 mg. per 100 cc., the chances were less than 50 per cent for obtaining a positive color reaction. When the concentration varied between 25 and 49 mg., the percentage of positive reactions increased to 70 per cent, and when the concentration was 50 mg. or over, the number of positive reactions increased to 100 per cent. The nitroprusside test, therefore, has definite limitations because of its failure to indicate the presence of β-hydroxybutyric acid and/or because of the presence of interfering substances in the urine when the concentration of total ketone bodies is below 50 mg.

The qualitative test made on the urine for determining the presence or absence of ketonuria does not reflect in any way the degree of ketonemia because the relationship is not strictly quantitative. This observation, however, does not detract from the practical value of the test in the diagnosis of suspected cases of ketosis or ketosis with complications because the qualitative determination of ketone bodies in the urine is a matter of considerable moment. Henderson[12] has assumed that any cow which shows a distinct positive test for ketone bodies in the urine has ketonemia.

METHODS

The animals were bled from the jugular vein into centrifuge tubes containing lithium citrate as the anticoagulant for the determination of plasma calcium, magnesium, inorganic phosphorus, chlorides and carbon dioxide. Potassium oxalate was used as the anticoagulant for the blood to be analyzed for ketone bodies, since the citrate ion is known to increase the total ketone bodies value, due to the formation of acetone by the oxidation of citric acid.

The methods for the analysis of plasma calcium, magnesium, and inorganic phosphorus were described in a previous publication.[8] The carbon-dioxide-combining capacity was determined by the Van Slyke and Cullen[24b] method, and the chlorides by the Van Slyke and Sendroy[24c] open Carius technic. The total ketone bodies in the whole blood and urine were determined gravimetrically by the Van Slyke and Fitz[24a] and Van Slyke[24d] methods, respectively. By these methods the acetoacetic acid and β-hydroxybutyric acid in the blood and urine are oxidized by dichromate and sulfuric acid with the formation of an insoluble mercuric sulfate addition compound. Therefore, the total ketone bodies represent both the acetone present as such and also that formed by oxidation. All other determinations were made by methods outlined in the *Official and Tentative Methods of Analysis*.[21]

In all cases the blood, urine, and milk samples were obtained as rapidly as possible.

DISCUSSION OF RESULTS

The experimental data secured from nine cows, three first-calf heifers, and three open heifers are summarized in tables III and IV. An examination of the data in table III shows that the ketone bodies in the whole blood in all but three of the mature cows and first-calf heifers on February 8 were definitely above the average values reported in the literature (table I). The lower values for the other cows, however, do not reflect their true condition. The two cows which freshened in December (488603 and 488607) had been severely affected prior to the beginning of this investigation and were recovering. The other cow (9440) received the benefit of the corn sugar and molasses and did not at any time show symptoms of the disease, although large amounts of the ketone bodies were being secreted in the urine and also detectable amounts in the milk.

It seems of importance to direct attention to the improvement, as evidenced by the marked reduction in the concentration of ketone bodies in the urine and blood, in all of the experimental animals during the time (February 8 to March 10) that they were receiving corn sugar or molasses and larger portions of the grain mixture with alfalfa hay. The individual values for ke-

TABLE I—Values for ketone bodies in the blood and urine and CO_2 in the blood for normal or apparently normal dairy cows as recorded in the available literature (expressed as volumes per cent for CO_2 and mg. per 100 cc. for ketone bodies).

Investigators	No. of Cows	No. of Detn.	Mean Value	Range		Remarks
				Min.	Max.	
Blood Ketone Bodies						
Sjollema *et al* (33, 35)	*	*	*	Traces		*
Little and Wright (15)	2	2	1.25	1.00	1.50	Milking cows
Christalon (6)	21	21	3.62	3.24	4.12	Nonpregnant cows
Christalon (6)	10	10	3.44	2.95	3.75	Nine oxen, one steer
Christalon (6)	27	27	4.28	3.04	5.54	Cows pregnant from 6 to 9 mo.
Sampson *et al* (28)	10	10	2.98	2.11	4.66	1-3 days after parturition
Boddie (3)	*	48	1.91	0.69	3.78	Various stages of pregnancy and lactation
Sampson and Hayden (29)	9	13	3.30	2.09	4.72	Late in pregnancy
Sampson and Hayden (29)	13	44	2.93	1.87	5.51	After parturition
Fincher (10)	1	1	4.16			After parturition
Average	93	176	2.99	0.69	5.54	
Urinary Ketone Bodies						
Sjollema *et al* (33, 35)	*	*	*	10.0	70.0	*
Little and Wright (15)	3	4	20.5	8.0	42.0	Milking cows
Carpenter (5)	2	34	16.0	6.0	32.0	Two fasting steers
Sampson *et al* (28)	6	9	7.83	3.45	13.15	1-3 days after parturition
Boddie (3)	16	16	3.28	2.50	6.37	Various stages of pregnancy and lactation
Sampson and Hayden (29)	7	10	7.17	3.22	13.55	After parturition
Fincher (10)	1	1	6.80			After parturition
Brouwer and Dijkstra (3)	8	12	26.8	17.0	37.0	Milking cows
Average	43	86	10.0	2.50	70.0	
Blood Carbon Dioxide						
Scheunert and Krzywanek (32)	7	7	62.8	52.4	68.9	5-15 years of age
Luchinetti and Neumayer (17)	100	100	67.2	52.1	80.5	Cows, steers and oxen
Luy and Thormählen (18)	1	10	61.5	56.1	68.5	7-year-old cow
Sampson and Hayden (29)	13	70	62.3	56.7	79.6	Pregnant cows
Average	121	187	64.9	52.1	80.5	

*No specific data given.

tone bodies in the blood of the cows at the time that the feeding schedule was initiated varied from 1.55 mg. to 79.05 mg. per 100 cc. (mean 23.09 mg.). It is unfortunate that urine samples were not available at the same time. Three days later, however, the urine values varied from 20.3 mg. to 549.5 mg. (mean 154.5 mg.) and the blood values ranged from zero to 43.88 mg. (mean 10.81 mg.). With the exception of four cows, all blood values were within the normally accepted range. By March 5, the average urine and blood values had decreased further to 53.6 mg. and 2.96 mg., respectively. The ketone bodies in the milk decreased to a mean value of 0.53 mg. and three cows were entirely clear. The same improvement also was noted in the values obtained for the first-half heifers and open heifers.

After March 10, when the ration reverted to the poor quality soybean hay, corn silage, and smaller portions of the grain mixture, the ketone bodies in the blood and urine became stationary or continued to decline in all but three cows. Nevertheless, detect-

able amounts of ketone bodies were being secreted in the milk in five of the six samples examined. These cows reflected the reduced intake of soluble carbohydrates and the lack of reserve carbohydrate by their inability to metabolize completely the ketone bodies, which was indicated by the rise in the ketone bodies in both the blood and urine (table III).

On April 29, it was again possible to secure urine samples from the entire herd, but only one blood sample was obtained.

TABLE II—Blood and urine values from cows suffering from acetonemia and acetonemia with complications as reported in the available literature (expressed as volumes per cent for CO_2 and milligrams per 100 cc. for all other values).

Investigators	No. of Cows	No. of Detn.	Mean Value	Range Min.	Range Max.	No. of Cows	No. of Detn.	Mean Value	Range Min.	Range Max.
Blood Ketone Bodies in Acetonemia						**Acetonemia Plus Complications†**				
Sjollema *et al* (33, 35)	*	*	*	*	100.0					
Sampson *et al* (28)	3	3	49.82	41.65	63.85	5	5	29.90	11.10	43.96
Hayden *et al* (11)	1		8.40			8	8	4.32	3.06	6.48
Lormore (16)	3	3	8.68‡	4.06	14.49					
Boddie (3)	3	3	9.01	7.08	10.06					
Sampson and Hayden (29)	5	5	44.56	27.93	63.85	29	45	5.26	1.71	30.30
Sampson and Hayden (30)	25	25	36.25	10.62	72.85	9	9	18.32	11.49	26.03
Fincher (10)	1	1	86.25							
Urinary Ketone Bodies in Acetonemia						**Acetonemia Plus Complications**				
Sjollema *et al* (33, 35)	*	*	*	*	1300.0					
Hayden *et al* (11)	1	1	29.1			5	8	8.44	4.48	19.48
Boddie (3)	1	2	77.2	38.1	116.3	2	2	39.61	10.46	68.75
Sampson and Hayden (29)	3	3	578.3	35.1	1209.7	35	35	18.65	3.62	267.9
Sampson and Hayden (30)	20	20	497.0	18.3	1309.2	2	2	55.10	11.40	83.79
Fincher (10)	1	1	1025.0							
Brouwer and Dijkstra (4)	8§	12	168.0	32.0	456.0					
Blood CO_2 in Acetonemia						**Acetonemia Plus Complications**				
Sjollema *et al* (33, 35)	*	*	*	35.0	*					
Sampson *et al* (28)	1	1	53.5			1	1	54.0		
Sampson and Hayden (29)	1	1	54.8			*	43	66.5	46.6	83.4
Sjollema (34)						*	31	58.8		
Blood Ca in Acetonemia						**Acetonemia Plus Complications**				
Hayden *et al* (11)	1	1	7.00			8	8	6.93	3.00	11.40
Sampson and Hayden (29)	5	5	8.83	7.60	9.50	51	51	6.78	2.37	12.50
Sampson and Hayden (30)	18	18	9.03	5.00	11.00	9	9	8.88	5.00	12.00
Sampson *et al* (28)						5	5	6.51	3.32	9.50
Blood Inorganic P in Acetonemia						**Acetonemia Plus Complications**				
Hayden *et al* (11)	1	1	6.02			8	8	3.87	2.65	6.48
Sampson and Hayden (30)	17	17	5.15	2.05	6.33	7	7	4.06	1.35	5.58
Sampson *et al* (28)						3	3	3.62	1.43	5.23

*No specific data given.
†Complications include one or more of the following: dystocia, metritis, hemorrhagic septicemia, pneumonia, dropsy, parturient paresis, malignant edema.
‡Expressed as acetone-calcium.
§Produced by feeding large quantities of silage of the butyric acid type.

TABLE III—Ketone bodies in the

TABLE III—Ketone bodies in the urine, whole blood and milk (expressed as mg. of acetone per 100 cc.) and the carbon dioxide content of the blood plasma (expressed as volumes per cent) of cows suffering from ketosis.

Cow	Calved	Urine					Whole Blood				Milk			Plasma		CO_2		
No.	Date	2–6	2–11	3–5	4–8	4–29	2–8	2–11	3–5	4–8	2–8	3–5	4–8	2–6	2–8	2–11	3–5	4–8
Mature Cows																		
488598	1-19-37		281.2	55.1	30.0	14.1	6.82	3.57	1.86	2.98		0.50	0.00	50.7	53.6	63.3	57.8	54.8
488599	12-29-36	255.5	136.4	14.4	23.1	71.0	35.00	20.77	4.19	20.98†		0.25		57.2	58.1	67.8	69.4	55.7
488602	12-24-36		32.2	76.2	17.6	14.6	21.50	1.09	6.20	5.83	4.96	0.25	11.65	59.8	63.6	64.2	64.8	53.0
488603	12-27-36		32.0	37.2	33.7	14.6	2.64	0.00	2.79	22.44		2.73	1.78		64.5	65.1	62.0	60.2
488605	12-26-36		177.4	46.6	15.4	00.0	25.27	19.53	1.24	1.24	42.90	0.00		47.1	65.9	70.5	64.8	68.3
488607	12-16-36		20.3	34.0	24.5	35.2	4.65	1.55	0.93	0.00		0.00	1.49		68.6	74.2	55.6	60.2
488608	12-23-36	77.8	111.0	15.6	11.4	35.2	31.16	6.97	3.26	1.49	8.18	0.99			43.5	66.0	55.1	62.0
94728	12-29-36		549.5	50.4	13.4	18.9	79.05	43.88	2.48	1.98		0.00	0.74		63.0	76.9	67.8	61.1
9440*	2-12-37		50.9	152.8	32.5	25.5	1.55	0.00	3.72	1.61		0.00	1.24		66.8	66.9	57.4	56.6
Average		166.7	154.5	53.6	22.4	25.5	23.09	10.81	2.96	6.50	18.66	0.53	2.82	53.7	60.8	68.3	61.7	59.3
First-Calf Heifers																		
9441	3-20-37			34.7	13.4	11.7	22.01		0.62	2.73			1.78		61.2	61.4	59.2	59.3
9444	3-19-37		53.4	61.3	49.6	23.1	3.41	0.78	3.26	17.86			0.50		65.8	67.8	61.1	62.0
9445	3-17-37			75.0	24.1	7.9			1.39	5.83			8.18				62.0	51.2
Average			53.4	57.0	29.0	14.2	12.71	0.78	1.72	8.81			3.49		63.5	64.6	60.8	57.5
Open Heifers																		
94726				124.7	19.1	15.4			2.32	0.00							60.4	58.4
495810			34.5	53.8	41.2	12.7	4.03	3.41	1.08	3.22					65.8	65.1	63.2	55.7
495811				47.4	14.6	7.4			0.00	0.00							65.0	57.5
Average			34.5	75.2	25.0	11.8	4.03	3.41	1.13	1.07					65.8	65.1	62.9	57.2

*Second calving.
†Acetone bodies value for 4-29 was 5.08 mg.

Between April 8 and 29, the owner reported a reduction in the milk yield and a gradual diminution in appetite, but the characteristic odor was not noticeable in the barn as it had been during the early part of February. It was also reported that the cows were not in as good condition as they had been during the previous month. The cows were turned out to pasture on April 28. The samples were collected during the late afternoon of the 29th and the results are recorded in table III. With the exception of three cows, the urinary ketone bodies were within normal limits. The total ketone bodies in the blood and urine of the most severely affected cow (488599) was 5.08 mg. and 71.0 mg., respectively.

The data summarized in table IV were collected to study the relationship of these constituents to ketosis in dairy cattle. In general, the values obtained for plasma calcium, magnesium and chloride do not show any significant deviation from the normal values and therefore do not reflect a disturbance in the mineral metabolism of the cows in this disease. The plasma calcium values are, however, definitely higher than those reported by the Cornell investigators.[11, 28, 29] The inorganic phosphorus values are slightly subnormal due to the restricted food intake and poor nutritional condition of the cows, and do not seem to be associated with ketosis *per se*. The plasma magnesium and chloride values are included as a matter of record, since values for these two constituents are not to be found in the literature in connection with ketosis.

The carbon dioxide values recorded in table III are also within the normal range for dairy cattle of the ages included in this study. Unpublished data at this station have shown variations as large as those recorded in table III in normal or apparently normal dairy cattle. An examination of the individual carbon dioxide values seems to indicate that severe ketosis is not necessarily associated with a marked change in the carbon-dioxide-combining capacity of the blood. This observation does not support the contention that acidosis is necessarily present in ketosis in cattle and is at some variance with those reported in the literature. This point, however, is being investigated further.

Brouwer and Dijkstra[4] produced an alimentary acetonuria in both apparently healthy and diseased cows by feeding large quantities of grass silage of the butyric acid type but failed to produce acidosis in any of the cows. Perkins, Hayden and Monroe[23] fed cows heavily on silage (corresponding to an intake of approximately 1.25 pounds of lactic and acetic acids daily) and found no evidence toward acidosis or that the concentration of ketone bodies was significantly higher in the urine.

In addition to the experimental data reported in tables III and IV, the specific gravity and total nitrogen content of the urine, and the calcium, phosphorus, magnesium, chloride, and total nitrogen content of the milk were also determined. These data do not show any significant deviation from normal; therefore, they are not of sufficient value to warrant inclusion here. Hutyra, Marek and Manninger[13] state, "The concentration of acetone in the urine eventually reaches a height at which the reaction becomes acid and the specific gravity falls to 1.005 or less." The specific gravity values which we found for the mature cows, first-calf heifers, and open heifers were 1.038, 1.042, and 1.039, respectively (range 1.020 to 1.052 for all animals). These values do not confirm the above statement in spite of the fact that some of the cows had ceased to ruminate and were severely emaciated.

It is the consensus of investigators that ketosis in cattle is due to a reduction in the catabolism of carbohydrates, the simultaneous oxidation of which is believed to be necessary for the complete oxidation of fat. Hutyra, Marek and Manninger[13] state that the disease is associated with a disturbance of the carbohydrate metabolism and consequent increase of fat catabolism and formation of excessive quantities of acetone bodies and cholesterin.

According to the prevailing theory, the cellulosic materials in the rumen of the cow, under the influence of bacterial fer-

TABLE IV—Calcium, inorganic phosphorus, magnesium and chloride content of the blood plasma of cows suffering from ketosis (expressed as mg. per 100 cc.).

Cow	Plasma Ca				Plasma Inorg. P					Plasma Mg.					Plasma Cl			
No.	2–6	2–11	3–5	4–8	2–6	2–8	2–11	3–5	4–8	2–6	2–8	2–11	3–5	4–8	2–8	2–11	3–5	4–8
Mature Cows																		
488598	11.0	10.7	11.3	10.1	5.48	3.90	3.56	3.93	6.35	2.13	2.15	3.04	2.65	3.50	331	337	339	337
488599	10.3	10.3	11.1	10.1	4.84	2.35	3.33	4.19	5.76	1.88	1.84	2.57	2.74	3.52	322	331	331	328
488602	10.0	10.8	11.0	10.7	5.39	3.01	3.56	4.19	5.51	2.31	2.21	1.76	2.45	3.00	316	337	344	328
488603		11.5	11.4	10.9		2.49	2.63	3.56	6.69		2.29	2.78	2.99	2.83	325	343	345	328
488605	10.1	10.7	10.7	10.1	4.04	2.67	3.62	3.68	7.23	2.24	2.38	2.01	2.49	3.50	316	316	331	322
488607		10.7	11.5	10.4		4.75	3.62	3.93	6.44		1.92	2.62	2.63	3.00	305	308	337	325
488608		10.9	10.7	10.7		2.67	1.79	3.06	4.84		1.79	2.99	2.59	3.45	339	348	345	341
94728		9.3	11.4	10.7		4.09	6.22	5.10	6.44		1.94	3.43	2.83	3.77	311	328	343	341
9440		10.5	11.3	10.5		4.34	3.45	4.62	4.56		1.68	2.11	2.75	3.65	358	358	352	322
Average	10.4	10.6	11.2	10.5	4.94	3.36	3.53	4.03	5.98	2.14	2.02	2.59	2.68	3.36	325	334	341	330
First-Calf Heifers																		
9441		11.2	11.4	10.5		5.08	4.98	4.84	5.71		1.56	2.55	2.45	3.31	358	367	373	322
9444		11.3	11.1	10.7		5.48	6.58	4.84	7.76		2.18	2.63	2.58	4.02	363	363	368	339
9445			11.8	10.1				5.84	7.23				3.47	3.38			376	316
Average		11.3	11.4	10.4		5.28	5.78	5.17	6.90		1.87	2.59	2.83	3.57	360	365	372	326
Open Heifers																		
94726			10.5	9.9				6.16	7.67				3.27	3.27			367	337
495810		10.7	10.8	9.9		5.98	3.01	5.00	6.07		3.92	3.71	2.55	3.50	337	363	364	331
495811			10.8	9.5				6.58	8.06				2.63	3.55			355	325
Average		10.7	10.7	9.8		5.98	3.01	5.91	7.27		3.92	3.71	2.82	3.44	337	363	362	331

mentation and enzymatic action, undergo hydrolytic cleavage into glucose and are assimilated as such. It has been shown by Markoff,[19] however, and demonstrated more convincingly by Pochon,[25, 26] Koffman,[14] and Woodman and Evans,[37] from *in vitro* experiments on the hydrolytic decomposition of cellulose by symbiotic bacteria from the rumen of the cow, that products other than glucose are also present in appreciable quantities, namely: acetic, lactic, propionic, pyruvic, butyric, hydroxybutyric, and valeric acids. The latter investigators were able to produce 3 Gm. of volatile fatty acids, of which 1.7 Gm. was butyric acid, from 10 Gm. of filter paper by fermentation with rumen bacteria.

Other investigators (Olson, Peterson, and Sherrard[22] and Berl and Koerber[2]), using cellulose-fermenting bacilli isolated from horse manure, have also demonstrated that the hydrolytic products of cellulose are volatile and nonvolatile fatty acids and alcohols. Schardinger[31] was the first to observe that acetone together with acetic and formic acids were obtained from carbohydrate fermentation. Reilly and coworkers[27] observed that acetic and butyric acids were apparently intermediate products in the formation of acetone and n-butyl alcohol in the fermentation of carbohydrate material. Koffman[14] has also indicated that acetone is formed in the rumen from the carbohydrates of the feed. Nicholson and Shearer[20] found much volatile fatty acid in the rumen of a cow that died from grass tetany and was autopsied immediately after death.

In view of the citations mentioned above, the presence of these acids in the fermentation products of carbohydrate materials is significant in the study of the physiology of ketosis in cattle. Of the simple fatty acids, Eckstein[9] and Deuel and coworkers[7] agree that propionic acid is able to function as a precursor of glycogen and glucose, while the even carbon fatty acids do not produce glycogen. Lactic and pyruvic acids are also definitely recognized as glycogen precursors. Since absorption from the rumen and digestive tract of the cow is taking place continuously, it is clearly of importance that any intermediate products that may arise in ruminant digestion must be taken into consideration. The fact that so many products of degradation may be present in the rumen indicates that additional glucose or quickly soluble carbohydrate material may be necessary at times completely to metabolize excessive amounts of fatty acids.

The results would seem to indicate that high-producing cows on ordinary roughage rations may be constantly demanding quickly soluble sugars to satisfy the demand for endogenous carbohydrate metabolism, which can not be supplied indefinitely through the catabolism of proteins and fats and diminishing deposits of glycogen, since the hay ordinarily used for stall feeding is usually low in sugars in comparison to pasture plants. This may account for the tendency for ketosis to occur more frequently in cattle during the winter months.

Since the completion of the work reported in this paper, the ammoniacal sodium-nitroprusside test has been applied to freshly collected urine samples from cows in the college herd. The test indicates that some degree of ketonuria, without clinical manifestations, is a more or less common condition among pregnant and lactating cows under stall-feeding conditions. This observation seems to suggest the possibility that ketosis may be manifested at various times. Acetonemia is usually associated with parturition, whereas ketonuria may occur at any time and may be of alimentary origin. The greatest incidence of this condition was found during the late winter and early spring months, after intensive stall-feeding, and before the herd was turned out to pasture. This is in accord with Boddie and Henderson.[12] The quantitative relationships between the amounts of ketone bodies in the blood and urine and the amounts of carbon dioxide and sugar in the blood of stall-fed cows are now being studied.

SUMMARY AND CONCLUSIONS

Some of the constituents of the blood, urine and milk were determined in samples collected from a herd of purebred Jersey

cows suffering from acetonemia. The disease had become so malignant that all of the milking cows, first-calf heifers, and open heifers were becoming affected. The ration consisted of poor quality soybean hay, corn silage, and a small amount of grain. The treatment consisted of changing the ration to alfalfa hay, corn stalks, larger portions of grain, and corn sugar or molasses, and the administration of chloral hydrate to the most severely affected cows. The condition was not entirely cleared up in some of the cows until after they were turned out to pasture.

The ketone bodies in the blood of the nine cows before treatment varied from 1.55 to 79.05 mg. per 100 cc. (mean 23.09 mg.) and the variation in the urinary ketone bodies was 20.3 to 549.5 mg. (mean 154.5 mg.). Four weeks later, these constituents had decreased to 2.96 and 53.6 mg., respectively. Ketone bodies in the milk varied from traces to 42.9 mg.

The values obtained for plasma calcium, magnesium, and chloride did not indicate a disturbance in the metabolism of these constituents in ketosis. The subnormal inorganic phosphorus values were associated with the poor nutritional condition of the cows. Acidosis was not encountered in this investigation and a lack of relationship was found between the blood-ketone bodies and the carbon-dioxide-combining capacity of the blood. A peculiar lack of relationship was also noted between the qualitative sodium-nitroprusside test and the amount of ketone bodies found in the urine by the quantitative determination. The qualitative urine test does not afford an accurate measure of the actual concentration of ketone bodies in the blood, but this observation does not detract from the practical value of the test in the diagnosis of ketosis.

In the light of recent investigations concerning the variety of hydrolytic cleavage products produced by the bacterial and enzymatic fermentation of cellulosic materials, it is suggested that these intermediate products may play an important rôle in ruminant digestion and particularly in the study of the physiology of ketosis.

The general conclusion is reached that certain practical measures can be taken to reduce the incidence of ketosis in farm herds under stall-feeding conditions. The most important of these are an improvement in the general management of the herd and better feeding, especially in regard to roughages.

The results also suggest that high-producing cows on ordinary roughage rations may be demanding additional quickly soluble sugars to satisfy their demand for endogenous carbohydrate metabolism.

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The Consumers' Milk Dollar

The cost of distributing fluid milk, which accounts for the difference between the price dairy farmers receive and that which the consumers pay, was analyzed by Mortenson, Witt and Heisig of the University of Wisconsin. The information was gathered from large and small firms engaged in distributing fluid milk and also from the state tax commission. The investigation was intended to answer the question, "Why don't farmers get a higher proportion of the consumer's dollar?"

The question is answered by the following figures for the ten-year period 1927-1936, which may be taken as a reasonable criterion:

	Cents
The consumer's dollar	100.0
Paid to farmers	56.0
Wages of workmen	17.6
Bottles, fuel, feed, etc	8.5
Salaries of officials	5.3
Total depreciation	3.6
Profit	1.9
Power, light and water	1.5
Advertising	1.3
Repairs	1.4
Taxes	1.1
Bad debts	0.9
Insurance	0.9

The author points out that dealers do not receive the full retail price of all milk delivered. Stores, hotels, restaurants, *et al* buy at lower rates. The large companies show a larger profit than the smaller ones. On that account, higher prices at the farm work a greater hardship upon the small dealer. (*N. H. Wis. Successful Farming, Sept. 1939.*)

The loss of vitamins in canned food is not serious, according to a report made by scientists at the American Chemical Society meeting in Boston (1939). Vitamins B, and C are destroyed to the extent of about 20 per cent, which is less than is lost in ordinary cooking.

The Relationship Between Salivary Output and Intestinal Effects with Carbaminoylcholine Chloride (Lentin)

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IN THE CLINICAL study of drugs which characteristically stimulate the secretory tissues and the musculature of the alimentary canal, it is usually necessary to base a conclusion regarding the effectiveness of a given administration upon such manifestations as salivation, defecation, and symptoms of abdominal discomfiture (colicky pains, increased borborygmi). While for many clinical purposes this information suffices, there are occasions when a more direct knowledge of the internal effects of a given dosage is desirable.

Approaches to this problem have been made by Nöll, using cats, and Bosse, on human patients, by means of x-ray examinations of the effects on intestinal motility under carbaminoylcholine chloride (Lentin). Although these experiments indicate quite clearly the doses necessary to elicit intestinal effects in the species used for the tests, they do not provide any means of determining the degree of effect in animal species which do not lend themselves so readily to roentgenological study.

The experiments herein reported were devised for the purpose of aiding in an evaluation of the intestinal effects not readily recognized by clinical examination. With a basis for correlating the salivary effects of a given administration with a degree of intestinal response established by experiment, the clinician can conclude how much intestinal action may be expected in a particular case from the degree of salivary activity. As the title indicates, the object of these studies was to establish such a basis, using unanesthetized dogs prepared with salivary and intestinal fistulas.

The stimulant action of carbaminoylcholine on the salivary glands, the smooth muscle, and the glands of the intestine is as well known as any phase of the wide choline effect characteristically stimulant to the parasympathetic division of the autonomic nervous system. For a review of the general literature on the physiology of the cholines the reader is referred to Dale[1]; more recent summaries on carbaminoylcholine are reported by Molitor[2] and Burgett and Bardens.[3] Table I presents a summary of a few pertinent observations from the literature on the actions of carbaminoylcholine chloride upon the alimentary tract of the dog.

EXPERIMENTAL PROCEDURES

Two mongrel male dogs, evidently normal in every respect, were prepared with Thiry-

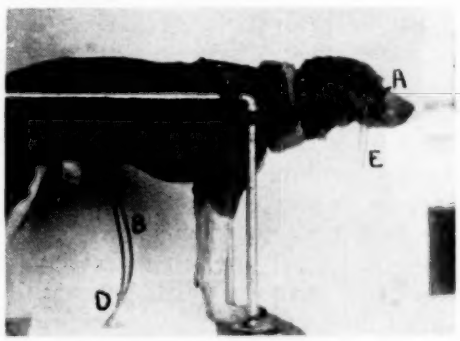

Fig. 1 (dog 1). This illustration shows the position in the stocks for experimental procedures and the attachment of apparatus for the collection of fistula saliva and succus entericus. The former consists of a special adapter (A) which is fitted to the skin around the fistula opening of the salivary duct and held in place by rubber bands attaching to a harness around the head; this leads to a graduated tube (E) in which saliva is collected and measured at five-minute intervals. The latter is a 30-inch length of one-fourth-inch rubber tubing, the ends (B) of which emerge from the openings of the Thiry-Vella loop (C) and lead to a beaker (D) in which succus entericus is collected so that it can be measured with a pipette at 15-minute intervals. The portion of the rubber tubing traversing the intestinal loop is fenestrated with holes to permit the secretion to pass into the tube.

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Vella loops, 8 to 10 inches long in the duodenojejunal region, and fistulas of the left Stenson's duct, after Glinski. The surgical procedures followed in the preparation of these fistulas were, in general, those recommended by Boldyreff.[4] Following their recovery from the effects of the operations, a period of several months elapsed before the present series was begun, during which time the animals underwent numerous control tests. Uniformity in the daily routine, including time of experimental handling, feeding and exercise, was practiced during the period of study. It is interesting to note that throughout the entire period of observation (several months), covering both the preliminary and the critical tests, there was never any evidence that they suffered physiologically as a result of the operations. In fact, both animals gradually gained weight. Apart from an occasional day of diarrhea or diminished appetite following a high dose of the drug used (these effects were not unexpected with the severity of the physiological responses), they continued in excellent health.

Early in the preliminary period each animal was trained to stand quietly in a specially constructed stall for a period of at least two hours. At the beginning of this time a fenestrated rubber tube was passed through the intestinal loop, both ends of the tube falling into a 250-cc. (8.3-oz.) glass beaker placed under the umbilical region (fig. 1). At five-minute intervals the beaker was replaced and the amount of intestinal juice collected during the preceding five minutes was measured by means of a pipette. Also, as a matter of routine, in the same intervals a current of air was passed through the tube from a rubber bulb, to preclude the accumulation of mucus blockage in the tube. Simultaneously with the collection of intestinal juice (= succus) but for periods of variable length, usually less than two hours, a special saliva collector was placed over the salivary fistula, where it was held in place by a rubber band around the muzzle and strands of rubber extending to a special head harness (fig. 1).

Actually, the amount of control saliva was scant; our results show that it never exceeded 0.1 cc. for a five-minute period. This conclusion was reached on the basis of more than a score of control studies, totaling over 200 single observations. In the majority of instances there was not sufficient saliva collected after a period of 30 to 60 minutes to form a drop in the collecting tube. Accordingly, as a criterion of saliva output, we set 0.1 cc. as the minimum. That is to say, for this study an output of less than 0.1 cc. for the five-minute period is regarded as normal.

Normal output from the intestinal loop offered a greater difficulty. From the start it was obvious that for every study a preliminary period of observation would be required, both to assure that the stage of exaggerated activity following the placement of the rubber tube had completely passed, and also to determine the actual basal rate of output at the time. A brief glance at the figures in table III will serve to illustrate the problem. While the output for the preadministrative periods is seldom constant, it may be compared with the postadministrative quantities to obtain a rough measure of the secretory effect on the intestinal mucosa (fig. 2). In making these comparisons it seems advisable to ignore the output from the first preadministrative period; the quantities here are usually quite excessive, probably largely the result of the mechanical stimulation afforded by placing the rubber tube.

The effects of carbaminoylcholine chloride upon the motility of the intestinal loop were studied in the same animals. Although these were experiments separate from the salivary-succus group, the conditions were the same as regards time of day, laboratory environment, method of administration, and other procedures. The only exception was the presence of a water-expanded balloon in the loop instead of the fenestrated rubber tube employed during the period of collecting intestinal secretion. Saliva was not collected during this series. The apparatus employed to transmit the effects of intestinal movements to a kymograph drum followed the general design of many that have been described. Briefly, it consists of a single toy balloon filled with

TABLE I—Citations from the literature: Dose levels of carbaminoylcholine (mg./kg. body weight) for various actions in the dog.

Salivation		Gastric Secretion		Gastric Motility		Intestinal Motility		Method	No. Animals		Investigator
Dose	Action	Dose	Action	Dose	Action	Dose	Action				
0.0025 0.005	None beginning			0.005 0.010	No vomiting Vomiting			Normal dog; subc.	1	1	Kreitmair[5]
0.1 0.25	None Profuse			0.25 0.50	No vomiting Vomiting	0.10 0.25 1.00	No defecation Defecation, purging	Normal dog; oral	1	1	
0.001 0.005 0.0075	Beginning Maximal effect							Normal dog; subc.	1	1	Nöll[6]
		0.002 0.008	7-fold+ 30-fold+ (Max.)					Parlow dog; subc.	1	1	
				0.0025 0.005	Increased Max. increase			Parlow dog; chloralose, subc. Parlow dog; subc.	1 1	1 1	Velten[7]
				1.5–2.0 2.5–3.0	Vomiting Vomiting			Normal pups; oral Normal adults; oral	8		Merke[8]
						0.010 0.250	Defecation Defecation	Normal dog; subc. Normal dog; oral	7		Molitor[2]

water and connected with a glass chamber, where changes in water pressure are converted into air-pressure variations which, in turn, are reflected on a tambour. The water level in the chamber was usually between 30 and 40 cm. (11.8 and 15.7 in.) higher than the balloon during the period of study.

The carbaminoylcholine specimens used in this study were supplied by the Merck Institute of Therapeutic Research, Rahway, N. J. All injections were given subcutaneously, in the cervical region, with solutions freshly prepared, employing the analytic balance and the volumetric flash.

Results

From the recordings presented in table III, it is apparent that the output of fistula saliva in no case was in excess of the normal rate (0.1 cc. for the five-minute period) with doses of carbaminoylcholine less than 0.004 mg./kg. With the dosage increased above this established minimal, the effect was augmented both as to the duration of the period of salivation and the total quantity of saliva collected during the entire interval of salivation. These two features of the reaction are signalized in table IV, where a correlation between the ratios obtained under "duration of salivation period" may be drawn with those from the column headed "quantity of saliva collected."

Obviously, the number of experiments is not sufficient to permit more than a tentative conclusion, particularly in that the figures represent the averages of values in some instances quite varied. (Table II shows the actual values.) Taking the results at hand, then, a means of estimating

the quantitative relationship between dose and effect is afforded in the data obtained where the three sialogogue doses were used. The ratio between the doses 0.004, 0.012 and 0.020 mg./kg. is of the order 1:3:5; the average duration of salivation with those doses, in the same order, shows 1:2¼:4; the average output runs 1:2¼:-3⅛. The difference between the dose ratio and those based upon recorded effects, although not a wide one, seems to point definitely to a factor of diminishing returns, as denaturing of the ester or exhaustion of the responding tissues.

How far these processes operate in precisely this manner is another question, according to the results from a single test with 0.040 mg./kg. in dog 1. With twice the maximum dose of the former series, the effects should fall short of double the

TABLE II—Output of saliva from left parotid glands.

Dog	Administration Subc.	Post-administration								
		1*	2	3	4	5	6	7	8	9
1	None	N	N	N	N	N	N†			
1	None	N	N	N	N	N	N			
1	None	N	N	N	N	N	N			
2	None	N	N	N	N	N	N			
2	None	N	N	N	N	N	N			
2	None	N	N	N	N	N	N			
1	0.3 cc. normal salt	N	N	N	N	N	N			
1	0.3 cc. normal salt	N	N	N	N	N	N			
1	0.3 cc. normal salt	N	N	N	N	N	N			
2	0.3 cc. normal salt	N	N	N	N	N	N			
2	0.3 cc. normal salt	N	N	N	N	N	N			
2	0.3 cc. normal salt	N	N	N	N	N	N			
1	0.001 mg./kg. Cc.C‡	N	N	N	N	N	N			
1	0.001 mg./kg. Cc.C	N	N	N	N	N	N			
1	0.001 mg./kg. Cc.C	N	N	N	N	N	N			
2	0.001 mg./kg. Cc.C	N	N	N	N	N	N			
2	0.001 mg./kg. Cc.C	N	N	N	N	N	N			
2	0.001 mg./kg. Cc.C	N	N	N	N	N	N			
1	0.004 mg./kg. Cc.C	N	2.9	0.5	N	N	N			
1	0.004 mg./kg. Cc.C	N	1.2	0.5	N	N	N			
1	0.004 mg./kg. Cc.C	N	0.7	0.2	N	N	N			
2	0.004 mg./kg. Cc.C	0.5	1.2	0.4	N	N	N			
2	0.004 mg./kg. Cc.C	0.2	0.5	N	N	N	N			
2	0.004 mg./kg. Cc.C	N	0.6	N	N	N	N			
1	0.012 mg./kg. Cc.C	2.9	7.7	3.3	1.0	N	N			
1	0.012 mg./kg. Cc.C	2.5	3.5	6.8	1.5	0.7	N			
1	0.012 mg./kg. Cc.C	3.0	8.2	0.5	1.3	0.3	0.3	N	N	
2	0.012 mg./kg. Cc.C	3.0	2.5	3.1	0.6	N	N			
2	0.012 mg./kg. Cc.C	1.6	1.9	2.4	0.8	0.1	N	N		
2	0.012 mg./kg. Cc.C	4.0	2.5	0.6	N	N	N			
1	0.020 mg./kg. Cc.C	0.5	8.8	2.9	4.2	4.4	1.9	0.1	N	N
1	0.020 mg./kg. Cc.C	1.0	9.4	8.5	2.6	0.2	0.6	0.1	0.3	0.1
1	0.020 mg./kg. Cc.C	1.0	4.4	3.1	1.0	0.1	0.3	0.1	0.1	N
2	0.020 mg./kg. Cc.C	0.8	3.2	2.9	2.7	0.6	0.4	0.4	0.3	0.1
2	0.020 mg./kg. Cc.C	0.8	4.1	4.8	1.6	0.5	0.2	0.7	0.2	0.2
2	0.020 mg./kg. Cc.C	2.1	4.5	3.8	1.8	0.4	0.1	N	N	N

*Periods of five minutes each; quantities in cubic centimeters.
†Less than 0.1 cc. (normal).
‡Cc.C = Carbaminoylcholine chloride.

corresponding maximums, provided that the same factors govern in the same manner.

Actually, saliva poured forth in excess of the normal rate for 2½ hours, providing 39.1 cc. (1⅓ oz.) in that period. Instead of twice the output, it was increased more than seven times; the duration of the effect was 3¾ times the preceding maximum.

In this one case not only do the relationships obtained with the smaller doses fail to hold, but they fail so utterly as to point definitely to a fundamental difference between the condition in effect under the two circumstances. It must be considered, of course, that in this latter case a very large dose was administered, involving severe physiological disturbances; symptoms of dyspnea and colicky pains were evident during the greater part of this period. Assuming that the marked circulatory depression, undoubtedly present during the course of this reaction, was to a large extent responsible for the excessive secretory effects, we introduced a factor which apparently did not operate in the former series to the extent of influencing the trend of relationship between dose and effect. The new factor, then, which we assumed to be a marked circulatory depression, appearing between the doses 0.020 and 0.40 mg./kg., distorted the decending ratio obtained for the smaller doses and reversed its order. Incidentally, it may be mentioned that in this case secretion from the intestinal loop was markedly increased for a period of one hour, or two fifths of the time that the excessive salivary action persisted.

On the whole, the effects upon intestinal secretion were somewhat more difficult to evaluate. The actual quantities collected at different intervals during the various tests are presented in table III. Attempting to facilitate the interpretation of these values, we established a curve for the average output by period from the three tests in which the same administration was employed. The graphs are presented as percentum variations, with the output for the second preadministrative period selected as the base. This was chosen over the first in order to reduce to a minimum the secretory effects of the mechanical stimulation consequent upon placing the rubber tube in the loop. A variable quantity of succus was excreted during these first periods, but usually this was considerably in excess of the output from the second. From that point, the general trend was either for the level

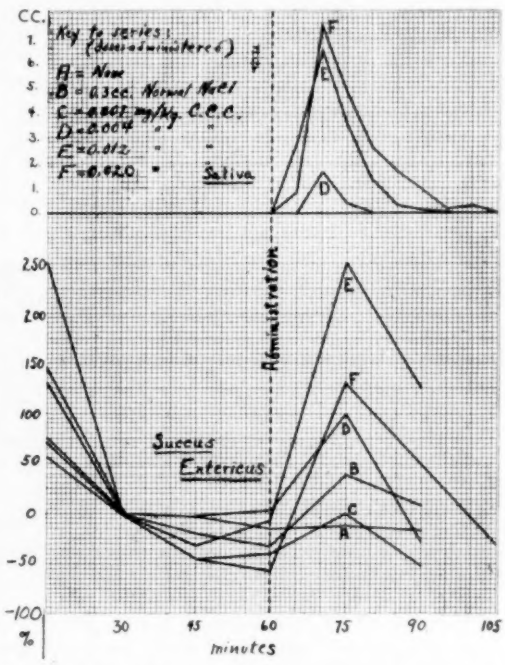

Fig. 2. Graph of secretory reaction in dog 1. The upper curves represent the output of fistula saliva (left parotid), and the lower, the output from the Thiry-Vella loop, collected simultaneously. Saliva recordings were made at five-minute intervals, less than 0.1 cc. being considered normal (0.0 cc.); each point represents the average output for the period, with reference to the time of administration, from three tests employing the same administration. Quantities in excess of the normal were observed only in the series D, E, and F during the post-administrative phase. Succus recordings were made at 15-minute intervals, with measurable quantities eliminated throughout the period of study; each point on the graph represents the average of three tests expressed as a per cent deviation from the basis established during the second preadministrative period. The actual quantities of secretion collected during the various experiments are shown in tables II and III.

to remain approximately the same, in some cases with slight variations, either as increases or decreases.

In the graphs obtained by this means (fig. 2) it is to be noted that with the controls and the dose 0.001 mg./kg. the post-administrative variations were relatively

TABLE III—Output of intestinal juice from Thiry-Vella loops.*

DOG	PREADMINISTRATIVE				ADMINISTRATION (SUBC.)	POST-ADMINISTRATION		
	1†	2	3	4				
1	11.4	3.4	5.4	4.6	None	4.7	4.8	
1	8.3	2.8	2.4	1.8	None	1.9	1.7	
1	11.3	4.8	3.1	2.8	None	2.9	2.9	
2	7.0	5.7	1.7	0.8	None	2.0	1.8	
2	6.0	3.7	2.2	1.7	None	3.3	2.1	
2	5.7	4.2	2.5	2.9	None	2.4	0.3	
1	2.6	2.8	3.7	4.4	0.3 cc. normal salt sol.	8.4	6.7	
1	5.6	4.0	1.7	0.6	0.3 cc. normal salt sol.	1.7	3.0	
1	10.2	5.6	4.0	2.8	0.3 cc. normal salt sol.	6.2	2.8	
2	7.0	2.0	1.3	2.1	0.3 cc. normal salt sol.	2.0	1.2	
2	5.5	2.6	3.7	4.9	0.3 cc. normal salt sol.	1.0	0.9	
2	8.0	5.0	4.3	3.3	0.3 cc. normal salt sol.	4.1	3.1	
1	8.2	6.2	2.1	3.4	0.001 mg./kg. Cc.C‡	6.5	3.7	
1	13.2	8.8	5.0	3.8	0.001 mg./kg. Cc.C	5.6	3.6	
1	8.0	2.5	2.5	2.9	0.001 mg./kg. Cc.C	5.2	1.1	
2	3.0	2.0	1.1	2.0	0.001 mg./kg. Cc.C	3.7	0.9	
2	4.6	3.7	4.2	3.1	0.001 mg./kg. Cc.C	0.6	0.4	
2	4.0	3.2	3.0	2.6	0.001 mg./kg. Cc.C	2.9	2.9	
1	5.7	4.5	4.2	4.1	0.004 mg./kg. Cc.C	7.6	1.5	
1	19.0	7.0	8.1	9.6	0.004 mg./kg. Cc.C	12.2	5.4	
1	9.0	3.3	2.0	1.3	0.004 mg./kg. Cc.C	9.5	3.9	
2	4.6	3.4	2.2	2.0	0.004 mg./kg. Cc.C	6.0	1.3	
2	3.8	2.4	2.2	2.3	0.004 mg./kg. Cc.C	7.8	2.1	
2	5.1	3.3	1.6	1.8	0.004 mg./kg. Cc.C	5.7	0.7	
1	11.4	4.0	3.2	5.0	0.012 mg./kg. Cc.C	16.5	9.5	
1	11.3	4.8	3.3	3.0	0.012 mg./kg. Cc.C	12.2	8.0	
1	6.1	2.8	1.4	2.8	0.012 mg./kg. Cc.C	12.8	8.8	
2	7.6	4.6	1.1	0.7	0.012 mg./kg. Cc.C	7.6	6.8	
2	7.0	3.0	2.3	1.2	0.012 mg./kg. Cc.C	9.9	1.3	
2	6.1	3.3	0.2	0.4	0.012 mg./kg. Cc.C	8.5	6.1	
1	5.7	3.7	2.1	1.2	0.020 mg./kg. Cc.C	11.8	9.6	5.2
1	6.0	3.2	1.8	1.9	0.020 mg./kg. Cc.C	16.1	8.7	4.3
1	13.0	10.5	5.3	4.5	0.020 mg./kg. Cc.C	12.3	7.6	2.5
2	4.7	3.2	3.6	0.8	0.020 mg./kg. Cc.C	6.5	6.9	4.2
2	6.1	5.1	4.1	0.5	0.020 mg./kg. Cc.C	7.5	7.3	5.3
2	5.6	3.1	0.9	0.9	0.020 mg./kg. Cc.C	7.3	6.7	2.9

*Same experiments as those recorded in table I.

†Periods of 15 minutes each; quantities in cubic centimeters.

‡Cc.C = Carbaminoylcholine chloride.

slight. With 0.004 mg./kg. there ensued a marked but transient increase in the intestinal output, whereas with 0.012 mg./kg. there occurred a further increase in the extent of the initial rise, together with a more prolonged period of excessive secretion. With 0.020 mg./kg., however, the initial post-administrative rise was not as great as with the preceding, although the period of increased output was more prolonged. Furthermore, upon computing the total post-administrative excesses on this basis, it becomes evident for both animals that whereas there is a marked increase as between the effects with 0.004 and 0.012 mg./kg., there is actually a decrease as between 0.012 and 0.020 mg./kg. The percentum average totals from the first and

second post-administrative periods for the doses mentioned are:

ANIMAL	DOSE (MG./KG.)		
	0.004	0.012	0.020
1	+63	+379	+279
2	+64	+172	+ 95

From a clinical point of view, at least, it appears that with the smaller doses, the therapeutic range, there is an optimal dose for stimulating intestinal secretions, variations from which, in either direction, result in a decrease in the total output.

a secondary drop below the control which gradually returns to that level; b) increased rate of the peristaltic waves, with or without a rise in the general level of the curve, followed by a reactionary period of subnormal frequence, and in some cases lowered levels; and c) prompt rise to unusual height, where the level is maintained, interrupted by sudden, rapid fluctuations, for a period relatively shorter than was encountered with the positive stage of the two preceding groups, and followed by a relatively long period of stagnation. This last type of response is illustrated by the curve in figure 3.

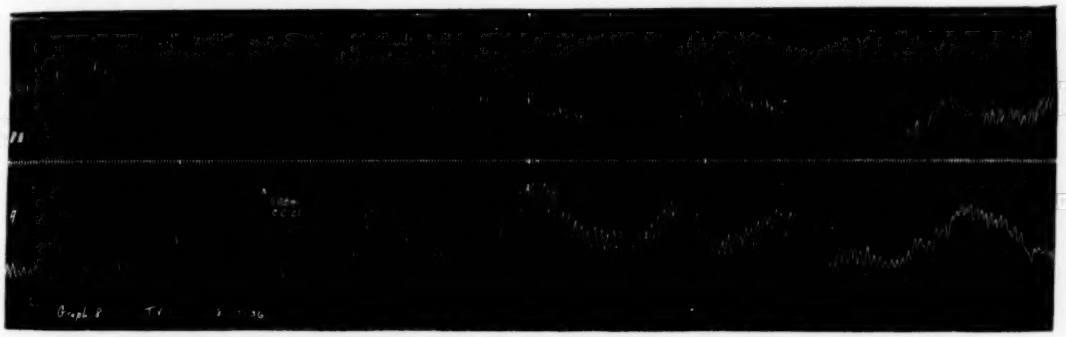

Fig. 3 (dog 1). Time: Two-second intervals. Administration: 0.004 mg./kg. carbaminoylcholine chloride subcutaneously. The lower record immediately precedes the upper. (A few seconds are required to adjust the drum. This interval represents the only lapse of time between the two recordings.) Records for a period of 45 minutes preceding the time of starting and 60 minutes following the completion of the recording show the following differences in the curve during the post-administrative period (period of salivation): 1) Higher general levels, 2) more frequent tone waves, and 3) greater amplitude of the tone waves.

The effects upon the muscular activity of the Thiry-Vella loops of carbaminoylcholine chloride in the doses 0.001, 0.004 and 0.012 mg./kg., subcutaneously, were studied in a series of tests in which the post-administrative curves were compared with those of the preliminary control periods. The general type of control was fairly uniform throughout the series; at least, the latter portions where the irregularities ascribable to the disturbances associated with placing the balloon had vanished. The variations observed following the administration of adequate doses seem to fall into three groups: a) No change in either the frequency or character of the long tone waves (peristaltic), with a definite rise in the general level for a variable period, and

Control injections of physiological salt solution were not accompanied by any alteration in the curve, as was the case with three out of four administrations of carbaminoylcholine chloride, 0.001 mg/kg. In a single instance (dog 2) a definite rise in the general level of the curve followed the injection of this dose and persisted for eleven minutes, followed by a period of subnormal level for about 13 minutes. Out of seven tests in which 0.004 mg./kg. was applied, four revealed the same type of reaction as the preceding (a), and one showed more frequent waves (b) for about ten minutes, with a secondary phase of about 20 minutes in which none of the typical long waves was recorded. The two remaining cases were attended by the third

TABLE IV—Summary of the effects of carbaminoylcholine chloride on saliva output.

CARBAMINOYLCHOLINE CHLORIDE (MG./KG. SUBC.)	PERIOD* OF EXCESSIVE SECRETION (MINUTES)			QUANTITY OF SALIVA COLLECTED DURING THE PERIODS* (CC.)		
	DOG 1	DOG 2	AVERAGE	DOG 1	DOG 2	AVERAGE
0.004	10	10	10	2.0	1.1	1.6
0.012	25	20	22.5	4.8	2.6	3.7
0.020	40	40	40	6.2	4.1	5.2

*Periods of five minutes each, during which 0.1 cc. or more of saliva was collected from the fistula. Quantities of less than 0.1 cc. for the five-minute period are regarded as normal. Each figure represents an average of three results.

type of response (c) with an active period of five minutes followed by about 15 minutes of relative inactivity. Of the four experiments employing 0.012 mg./kg., three were accompanied by the latter reaction (c) and one by the second type (b). It may be added that although the active period was longer where the second type of reaction occurred, as compared to those with the third type, neither the apparent force of the contractions nor the duration of the effect differed between the doses 0.004 and 0.012 mg./kg. A single administration of 0.020 mg./kg. was followed by (b) increased rate of peristaltic waves, for about 20 minutes, with a moderate rise in the general level for a portion of the same period.

TABLE V—Summary of the effects of carbaminoylcholine chloride on the motility in the Thiry-Vella loop.

TYPE OF RESPONSE	MG./KG. SUBC.				
	CONTROL	0.001	0.004	0.012	0.020
No change	4	3	0	0	0
Tone increases (a)	0	1	4	0	0
Peristaltic increase (b)	0	0	1	1	1
Long tone wave (c)	0	0	2	3	0
Totals	4	4	7	4	1

These results are summarized in table V.

SUMMARY

Subcutaneous administrations of carbaminoylcholine chloride (Lentin) in graded doses to two unanesthetized dogs, each with a fistula of Stenson's duct and a Thiry-Vella loop, revealed secretion and motility effects in the following relationships:

1) Parotid secretion was augmented with 0.004 mg./kg., while 0.001 mg./kg. was without that effect; with increases beyond this minimal effective dose the output was further augmented both as to duration of the flow and total quantity excreted.

2) Intestinal secretion similarly was definitely increased beyond the preëxisting rate by 0.004 mg./kg., 0.001 mg./kg. being apparently inadequate. The increase was greater yet with 0.012 mg./kg., whereas 0.020 mg./kg., while evoking a period of maximal duration, failed to augment either the rate of output or the totals which accompanied the smaller doses. Accordingly, of the three effective doses, 0.12 mg./kg. in these experiments was accompanied by the largest output, but not the most prolonged action.

3) Intestinal motility, although perhaps mildly stimulated in one out of four cases where 0.001 mg./kg. was administered, was definitely augmented by 0.004 mg./kg. Furthermore, doses three times greater were accompanied by curves which could not be distinguished from those of the former (0.004 mg/kg.) either in extent or duration of excursion.

From these results it is reasonable to conclude that in the dog the smallest dose of carbaminoylcholine chloride with a definite sialogogue action is also the smallest dose which augments motility and secretion in the small intestine. With dose increases

beyond this minimal, within the therapeutic range (ca. 0.004 to 0.020 mg./kg.), the salivary effect, both as to the period of salivation and the total output, varies directly with the dose. On the other hand, the smooth muscle and glands of the intestinal wall do not appear to respond to the same extent to the same influence, since the records reveal a maximal output of intestinal juice and maximal contraction curves with submaximal doses.

While only a single experiment was carried out with a very large dose, it is evident from the results that the relationship in this range between size of administration and degree of effect differs from that established for the therapeutic range.

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Local Treatment of Dog Bites

It is customary for physicians to cauterize the abrasions and wounds caused by dog bites with fuming nitric acid. The object, of course, is to kill any rabies virus the wound may contain. But, since particulate material deposited in a wound may be carried away quickly by the lymphatic circulation, at least in part, cauterization is thought by outstanding physicians to be of doubtful value. Certainly, there is no logic in depending exclusively upon cauterization and depriving the victim of the additional protection conferred by rabies vaccine.

Opinions differ as to whether a wound caused by the bite of a dog should be treated precisely as one would go about disinfecting any contaminated wound or whether it should be pounced upon forthwith with a vicious, tissue-killing acid. Because the virus of rabies causes a serious disease does not change the principles of local wound disinfection. Beyond the cauterized level are devitalized tissues caused by the caustic which favor rather than inhibit the propagation of pathogens. It is not likely that rabies virus is an exception. From our many experiences in the treatment of dog bites in animals it seems reasonable to agree with the "anticaustic" group of physicians. The cauterization of the dog bite is a far-fetched idea handed down from the dark ages of medicine.

Nicotinic Acid

The companionship established between blacktongue and pellagra by Goldberger led to the discovery that vitamin G is a complex mixture of vitamins and not identical with the P-P (pellagra-preventing) factor of food. Though Funk (1912-1913) discovered nicotinic acid in rice polishings, no importance was attributed to it until Elvehjem (1938) found that this acid and its amide in liver extract is anti-blacktongue and probably a cure for pellagra.

Though closely related to the highly toxic alkaloid of tobacco, nicotinic acid has been found to be a hundred times less poisonous to laboratory animals than nicotine. It is found in liver, lean meat, milk, cod fish, brewers' yeast, whole wheat, kidney and many vegetables, and is probably not synthesized in the body, since symptoms of disease in certain animals develop when it is not contained in the diet.

The curing of blacktongue with nicotinic acid or its amide is dramatic, as was shown by Elvehjem, and being presumably a constituent in metabolism of all animals, many other uses may be found for it. It is thought by good authorities to be essential to normal functioning of the digestive tract, skin and nervous system. The amount required to prevent blacktongue in dogs weighing 5 to 10 kg. (11 to 22 lbs.) is 1 mg. per day (.015 gr.). *(B. W. Fairbanks and Elizabeth Curzon. Nicotinic Acid. The North American Veterinarian, x, Nov. 1939, pp. 17-21.)*

In the science of advanced histophysiology there are classified over 17,000 nerve filaments for the spinal cord alone.

Canine Leptospirosis in the United States*

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IN 1937, Dr. S. T. Michael of the Hospital of the Society for the Prevention of Cruelty to Animals solicited coöperation in the diagnosis of a highly fatal disease of dogs which was observed with increasing frequency in San Francisco. At first, only cadavers and, later, very sick or moribund animals were brought to the laboratory. Since clinical observations could not be made in the majority of cases, the study was confined to autopsies, cultures and, later, to serological examinations.

The nature of the early specimens and the lack of experience made the investigations rather tedious and often discouraging. However, a microscopic study of the kidneys and the subsequent isolation of Leptospirae established conclusively the existence of canine leptospirosis in California. The examination of serums from various parts of the United States attested to the wide distribution of this malady. In order to give benefit and to encourage further inquiries in clinics, the experiences—pathological, etiological, diagnostical and epizoötiological—are herewith detailed and critically compared with the studies made in Holland and elsewhere.

AUTOPSY FINDINGS

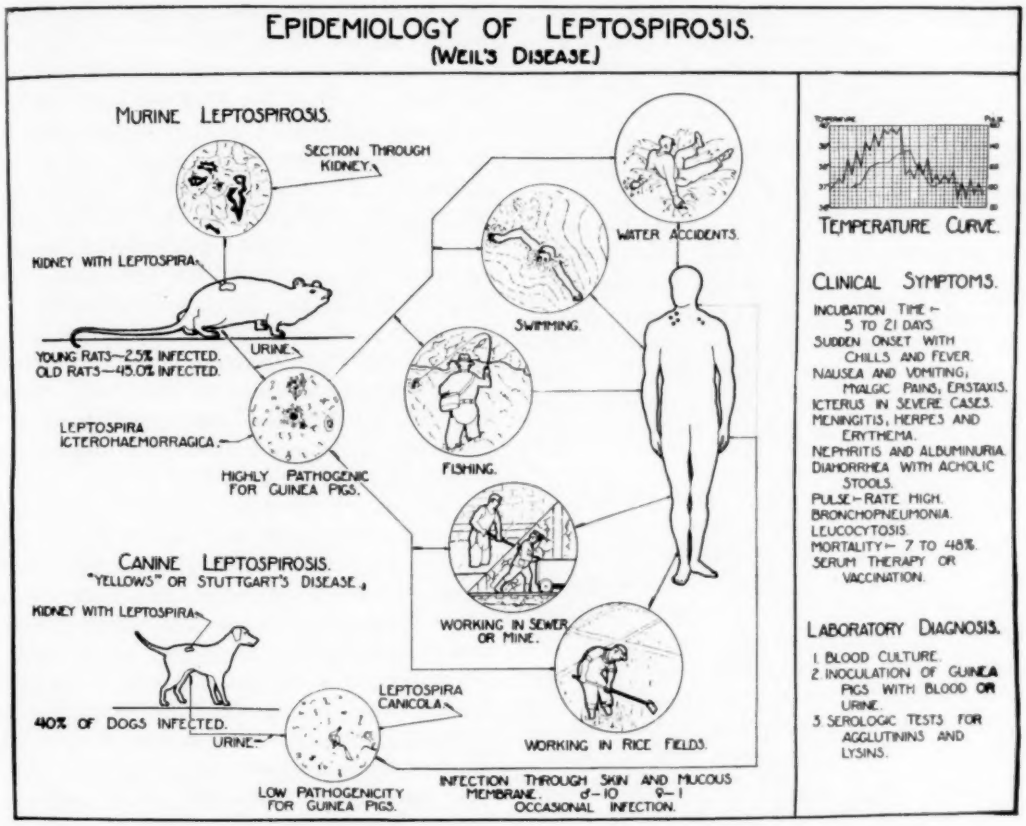
The gross anatomical findings on the first 15 dogs clearly indicated two distinct disease entities; subsequent observations merely confirmed the original interpretation. Of 88 dogs autopsied by the laboratory, 41 presented a jaundice in varying degrees; they are classed as the "icteric type." Forty presented predominantly a profound hemorrhagic gastroenteritis with little or no icterus; they are recorded as the "hemorrhagic type." Transition stages between the two forms were seen in seven dogs. Apparently they had passed through the hemorrhagic type but died in the icteric stage.

The icteric type resembled the so-called "yellows," or Weil's disease or infectious jaundice of dogs. Most of the animals were in a state of coma or dead when received. As a rule, the cadavers revealed a readily recognizable icteric discoloration in the nonpigmented, hairless areas. It was more pronounced in the subcutaneous, fascial and visceral tissues.

The jaundice varied in intensity from a pale yellow to a deep orange. The conjunctivae were invariably coppery red, the tongue and gums of the mouth were covered by yellowish crusts, and small erosions were present on the buccal linings in some animals. The spleen was either normal or subnormal in size, dry and covered by a wrinkled capsule. The liver, normal in size or slightly enlarged, was somewhat pulpy, clay or saffron colored and congested; invariably the biliary passages were patent, but the portal lymph nodes were succulent and sprinkled with hemorrhages. Serous petechiation covering the pleural and peritoneal lining was always present. The intestinal tube, in particular the stomach mucosa, was injected, and in varying stages of a catarrhal process, as a rule, free from biliary pigment. Aside from occasional hemorrhages in the renal cortex, these organs exhibited slight parenchymal changes. The entire respiratory tract was usually injected, the lungs dark brown, voluminous on account of terminal edema, spotty or diffuse pneumonic infiltrations and hemorrhages.

The findings correspond in general with those recorded by Klarenbeek and his coworkers in Holland; Pugh and others in England; Wirth in Vienna; and Uhlenhuth and Zimmerman in Germany. However, in one respect, a noteworthy difference must be emphasized. In the California cases the spleen was never enlarged. In fact, the dry, anemic, atrophic structure was a constant finding. It is not unlikely that the acute splenic tumor mentioned by previous

*From the George Williams Hooper Foundation, University of California.



observers is associated with *Leptospira icterohemorrhagiae* and not with *Leptospira canicola* infection. Although the autopsy reports on human classical leptospirosis relative to the enlargement of the spleen are contradictory, it is important to note that in uncomplicated Weil's infections the organ is of normal size. Furthermore, it is recalled that the spleen is not changed in experimental infections.

The hemorrhagic type resembles canine typhus or Stuttgart disease. The cadavers were dehydrated; the mouth and anal openings were soiled. The mucosa of the buccal cavity was, as a rule, dry, covered by diphtheroid, necrotic erosions and ulcers which were also present on the tongue and the margins of the lips, and were invariably accompanied by foetor exore. The viscera were dark and presented a hemorrhagic inflammatory process of varying intensity. The mucosa of the stomach and duodenum and the colon and rectum was infiltrated, and dark purple or grayish to dirty brownish; in the pyloric region thick, gelatinous folds were layered on a hemorrhagic submucosa. The blood-tinged, tar-like content emitted a strong ammoniacal odor. All lymph nodes were edematous and hemorrhagic.

The spleen, as a rule, was small. Occasionally it was studded with areas of hemorrhagic engorgement of varying size; typical infarction was not noted. The liver was mottled, quite hyperemic and friable. Hyperemia and streaking of the renal medulla were always present. Petechiation of the pericardial covering, auricles and the costal pleura and, occasionally, diffuse effusions and myocardial and pulmonary hemorrhages were noted. Focal lesions on the costal pleura, a necrotizing pleuritis considered as a characteristic manifestation of canine typhus, were not observed.

The kidneys were slightly enlarged, hyperemic and soft with moderate parenchymatous changes indicative of the acute febrile process. The central nervous sys-

tem showed no gross lesions, except general hyperemia.

Bacterioscopic Examinations.—The liver, spleen, blood, urine and kidney specimens revealed in stained preparations a variety of bacteria. Fresh cadavers were searched with the aid of wet preparations by darkfield illumination. At first, a startling number of motile filaments were mistaken for Leptospira. Probably every beginner experiences difficulties in the differentiation of true Leptospira from film filaments which appear as precoagulation stages. True spirochetes are actively motile, possess terminal hooks and a definite bead-like structure.

Fennell and Hall detail their experiences and misgivings, while many years ago Noguchi and Schüffner discussed and devised means which are of assistance in avoiding those errors so commonly committed by the inexperienced. In this connection, it must be emphasized that it has not been possible to demonstrate the Leptospira conclusively in blood films examined by darkfield. Even repeated centrifugation, as advocated by A. Ch. Ruys for human blood and examination of the blood plasma of moribund dogs, has not given trustworthy findings. Darkfield examinations of the spleen and kidneys offered suggestive leads and should be used when suitable specimens are available. However, all the microscopic examinations should be checked by cultures or animal inoculations. Provided the cadavers were fresh and the blood cultures proved sterile, the enrichments of the heart blood in the special media were held under continued observation. It is regrettable that only a limited number of autopsies were suitable for such examinations.

In the majority of instances a search for Leptospirae was made in properly fixed tissue blocs with the aid of the silver staining methods. Here again, experience taught certain pertinent details generally overlooked or not sufficiently stressed by previous observers. The technic outlined in appendix A proved quite trustworthy and requires no gold toning. Pieces of the organs were also fixed in Zenker-formol solution and the sections stained with hematoxylin-eosin.

Leptospirae were readily demonstrated in the kidneys of 19, or 48.6 per cent, of the 39 jaundiced and of 24, or 66 per cent, of the 36 hemorrhagic types of the malady examined by the silver method. Six of the seven dogs, which suffered from both types of the infection, gave positive Leptospira findings. In every instance, the spirochetes were found in the tubules of the kidneys, singly or in clusters. Subsequent examinations of dogs in the early stages of yellows showed the Leptospira in the blood stream and spleen; thus the absence of the organisms in nearly one-half of the kidney section is in part explainable.

Capillary engorgement with extravasation was quite uniform in the renal tissues of the dogs with positive Leptospira findings. Desquamation of the degenerated renal epithelium in the convoluted tubules and enlargement of the glomeruli associated with distention of the tuft was quite common. The degree of the degenerative changes both in the glomeruli and the tubules was, however, quite variable. In a few specimens, the areas of necrosis and hemorrhage were surrounded by round cells and fibroblastic proliferations.

Moderate dissociation of the liver structure through hemorrhages or perivascular edema was accompanied by extensive degeneration and partial necroses in the periphery of the lobules. Pigmentation in the form of finely granular bile pigment was present in the jaundiced cases, while fatty degeneration of the Kupffer's cells, with little pigmentation, predominated in the hemorrhagic types. The icterus was in no way associated with blocking of the biliary passages. It was, however, of hepatogenic origin. The splenic substance was reduced and in some instances showed depletion of the malpighian corpuscles.

Hemorrhages, erythrocytophagia and moderate pigmentation varied from case to case. Diapedesis of the red cells accompanied the dilatation of the capillary beds in the lung. Alveoli surrounding the bronchioles were frequently filled with serum, polyblasts and desquamated living cells. These areas of gray hepatization were of

an alveolar, miliary or lobular distribution. They were associated with various microbian findings and were doubtless of a secondary nature.

CLINICAL OBSERVATIONS

From the notes furnished by Dr. S. T. Michael supplemented by personal observations, we recorded the clinical course of the two types of infections.

Icteric Type.—The onset of the illness is, as a rule, quite sudden. In some instances the jaundice appears as the first symptom. It is particularly evident on the sclerae and the unpigmented portions of the skin and may vary from a lemon to a deep orange-yellow color. The temperature varies from 102.5° to 104° F.; it rarely reaches the level of subnormal, as is quite common in the hemorrhagic type.

The dogs when received at the hospital are listless, may eat sparingly, may vomit and they are frequently constipated. The urine is dark yellow and quite scanty and the albumin content is high. In a variable percentage of dogs hemorrhages develop on the skin of the minor surfaces of the legs and shoulders. Superficial erosions and hemorrhages may be noted on the gums and tongue. There may be marked emaciation. Usually, the odor of the animal is repulsive and in some cases definite muscular tremors are noted. In the few cases in which recovery was observed, the convalescence was rapid.

Hemorrhagic Type.—The onset is sudden in this type. A dog may be perfectly well the night preceding its illness. Weakness in the posterior extremities, refusal to eat the morning meal and a mild depression are noted. If the temperature is taken, it will be found to be rather high (103-105° F.). There is also present in some cases a mild congestion of conjunctiva (only very vigilant owners bring their animals to a veterinarian at this stage).

On the second day one finds in the majority of cases a rather marked drop in temperature from 103-105° to 100-102° F. The mental depression is more pronounced and the animal is quite apathetic; there is dyspnea, continual anorexia and marked thirst. Occasionally, persistent vomiting takes place. The buccal membranes, when examined on the second or third day, may show at first irregular, congested patches which later become hemorrhagic or dry and necrotic. The gums are covered by a stringy saliva and emit an offensive odor. The difficulties in swallowing are due to a pharyngitis. In more advanced cases intense depression, dehydration, muscular tremors, abdominal pains on palpation, bloody vomitus, actual epistaxis and bleeding gums, and even necrosis of the tongue have been observed.

Since seromucous plugs are frequently present, the disease may be confused with distemper. As the disease progresses, striking and rapid emaciation and dehydration leading to a dry skin, muscular tremors, and eyes with injected conjunctiva sunken into the orbits become prominent. The temperature is subnormal and the pulse accelerated, arhythmic, small and thready; the respiration regular and deep. Petechial hemorrhages may be seen in the skin of younger dogs. Usually, the animals are constipated, or blood-tinged, liquid stools may be passed involuntarily.

The course is commonly rapid. Dogs develop the symptoms just described in quick succession and drop into deep coma or develop clonic-tonic convulsions and die in from three to ten days. Recovery is seen more frequently in young dogs.

In San Francisco, the hemorrhagic type is seen predominantly in adult dogs. Only too often this malady is confused with blacktongue, from which it may be differentiated by its rapid course, vomiting and great depression early in its course. It is unquestionably important that in the future the serum of the so-called cases of blacktongue be subjected to lysis tests in order to establish some criteria relative to the extent of canine typhus and blacktongue.

The decidedly limited and rather selective observations amply support the consense of small animal practitioners that nearly every case of acute jaundice of the dog is doubtless caused by the Leptospira.

ETIOLOGICAL INVESTIGATIONS

In view of the conclusive demonstration of Leptospira in the renal tubules, no effort

was spared to isolate the organism. It was particularly desirable to have pure cultures in order to determine the possible relationship of the variants of Leptospira to the two clinical and anatomical types of the infection. On the basis of many years of experience and observation, Klarenbeek concluded that the differences in the characteristics of the canine infectious liver injury or renal damage are induced by different strains of Leptospira. Furthermore, at the time these studies were undertaken, neither cultures nor serological findings on canine leptospirosis had been reported from the United States.

ANIMAL INOCULATIONS

Since the blood or the organs of the first 60 dogs submitted for autopsy were, as a rule, contaminated with intestinal organisms, the early attempts at isolation of the Leptospira had to be confined to intraperitoneal inoculations of guinea pigs, rats, mice and rabbits with suspensions of pulped spleen, liver and kidney fragments. These preliminary experiments proved unsuccessful. The animals either remained well or died from *Clostridium welchii* or *Escherichia coli* infection.

On the advice of Dr. Linda B. Lange of the Johns Hopkins School of Public Health, young guinea pigs weighing 50 to 100 Gm. were then chosen as experimental animals. Instead of daily temperatures, which revealed nothing of significance, the weights of the animals were carefully watched. Generally, in nine to twelve days the guinea pigs were sacrificed and cultures of the heart blood and peritoneal fluid were prepared. Gross lesions were rarely apparent. The organs were scrutinized for Leptospira in the darkfield and in sections stained by the silver method, and emulsions of the liver and kidneys were passaged through three to five series of guinea pigs. On the third to fourth passage the animals lost weight and, when sacrificed, presented a distended gallbladder, congested viscera and injected inguinal lymph nodes. Only in one instance were the subcutaneous and fascial tissues icteric after the injection of the organ emulsions.

Repeated passage leads sometimes to a fatal infection, death usually occurring on the 9th to 15th day. Dhont *et al* and Uhlenhuth experienced the same difficulties in the isolation of the canine Leptospira by the guinea pig-inoculation method, and perusal of the literature indicates that the spirochetes associated with the dog infection are of a very low pathogenicity for rodents. Since fatal infections are rare, Wirth advocated the withdrawal of peritoneal fluid on the third or fourth day after the injection of the organ emulsion. He claimed that quite often he had seen Leptospirae in the darkfield preparations and, thus, had been able to secure cultures on suitable media with ease. This procedure was tried with success in two instances.

In retrospect, the experiences collected during the many futile attempts to isolate the Leptospira from dogs are amply supported by the observations of Syverton, who infected over 800 guinea pigs with 34 strains of Leptospira. Although he recorded fever in 80 percent of the animals infected, icterus was seen in only 1 per cent of the guinea pigs. A great deal of time and disappointment could have been avoided if it had been more generally known that Leptospira of canine and even human or rat origin may not cause fatal infections in guinea pigs at first, and that serial passages are an absolute necessity. Moreover, it should be emphasized that success may ultimately be achieved if young, preferably nursing, guinea pigs are used.

In the light of recent reports by Packchanian, it appeared probable that certain species of field mice might prove more susceptible for canine Leptospira than the guinea pig. Preliminary tests with a limited number of *Peromyscus maniculatus, P. truei, P. musculus mordax* and Diplodymus (?) trapped in California and injected with freshly isolated strains of Leptospira from the blood of dogs or the urine of a human infection were decidedly disappointing. Only one *P. maniculatus* injected with a culture died on the sixth day with lesions of icterus and pulmonary hemorrhages. Serial passages through three to four mice failed to enhance the pathogenicity. Sec-

tions and cultures of the animals proved entirely free from Leptospira.

Strains of *P. polionotus*, *P. eremicus* and *P. maniculatus*, which were kindly sent to us by Dr. A. Packchanian and recognized by him as uniformly susceptible for Leptospira, proved entirely refractory. The mice remained well and at autopsy gave sterile cultures, and a painstaking search for spirochetes in sections failed to reveal the organisms. On a comparative basis, the young guinea pigs still proved to be more useful for the isolation of the Leptospira than the various American deer mice.

TABLE I—Methods successful in the isolation of 15 L. canicola cultures.

STRAIN	BLOOD	ORGANS	URINE
Santa Rosa	Direct culture and guinea pig passage	—	—
D56	Direct culture	—	—
D62	Direct culture	—	—
D64	Direct culture	—	—
D65	Direct culture	—	—
D72	—	Guinea pig passage (3) kidney suspension peritoneal fluid	—
D90	Direct culture from blood clot	—	—
D94	Direct culture from blood clot	—	—
D95 (living)	Direct culture from blood clot	—	—
D96 (living)	Direct culture from blood clot	—	—
D97	—	Peritoneal fluid and liver of guinea pig inoculated with kidney material	—
D110	Direct blood culture	—	—
D113	Direct blood culture	—	—
D115	Direct blood culture	—	—
Morenzoni (human)	—	—	Guinea pig inoculated with urine

CULTURAL STUDIES

Enrichment or plating of blood, urine and suspensions of organs on the usual culture media either proved sterile or so grossly contaminated with intestinal microörganisms, which had invaded the tissues before the cadavers were received at the laboratory, that the use of special elective media for Leptospira appeared futile. Various special culture fluids, as recommended by Noguchi, Fletcher and others, were tried with little success even with blood specimens secured before death, and proved sterile in broth or beefheart media. Comparative studies proved the medium described by Verwoort and modified by Schüffner as the most satisfactory for the isolation of the canine Leptospira. The composition and preparation of the medium is presented in appendix B.

The culture fluid compounded by Korthof is also quite useful. For routine examinations and particularly for the preparation of antigens, the Verwoort medium is readily made and has excellent keeping qualities.

In the course of the preliminary trials, it has been noted that citrated blood frequently yielded no growth while blood defibrinated with glass beads has proved uniformly dependable. The first culture was obtained from an icteric dog about 24 hours before death. In practice, 5 to 10 cc. of blood is withdrawn from the femoral or jugular vein; it is expelled directly through the rubber stopper into a sterile vaccine or serum vial with glass beads and rotated until completely defibrinated. Small quantities, 0.2 to 1 cc. of the blood, are then added to 3 cc. of Verwoort medium.

The tubes are incubated at 32 to 35° C. At intervals of five to six days transplants are made to fresh tubes. All specimens are held under observation for five to six weeks, since observations have shown that growth may not appear until that time. Progressive growth is controlled in darkfield illumination with a Zeiss 40 (0.65; 0.17) or Leitz 3 (2/3 inch) and 6L (1/6 inch) objective.

During the past year, blood recovered at autopsy has been used; in fact, broken clots may be successfully cultured, since it is known that the organisms remain alive for some days in blood which is free from bacteria. The various procedures which led to the isolation of 14 cultures from an equal number of dogs are listed in table I.

A casual comparison of the results recorded in table I with the total number of dogs examined might leave the impression that the San Francisco achievements are not very encouraging. If one recalls that Esseveld in Holland isolated 17 strains of Leptospira from 17 dogs, the cultivation of 14 strains from 60 dogs calls for an explanation.

As repeatedly stated, the animals were sent to the laboratory in a moribund state at a time the owner could be assured that the dog would succumb to the infection, and the hospital was at liberty to dispose of the pet. Uncontaminated blood or organ specimens were, therefore, rarely obtainable. In particular, dogs suffering from the hemorrhagic type or canine typhus were not secured at a time when the blood stream was free from intestinal invaders. Consequently, Leptospirae have thus far been isolated only from definitely jaundiced dogs. Experience has taught that at a time when the veterinarian suspects canine typhus and collects blood, the specimen is invariably contaminated and useless for Leptospira cultures. In properly organized dog hospitals, cases for bacteriological examinations are frequently available and cultures in the early stages of the disease may be readily obtained. Information along these lines should be collected for reasons to be discussed later.

A study of the published findings on dog leptospirosis by Klarenbeek, Roos and his associates conveys the impression that one form of the disease seen in Holland is accompanied by persistence of the spirochetes in the kidneys, and that a leptospiruria is a rather frequent sequel of the infection. A limited series of attempts in San Francisco to isolate the Leptospira from the urine of dogs were not successful. Catheter specimens of urine were inoculated into guinea pigs or cultures were prepared. For these tests the urinary sediment, after prolonged centrifugation, was used. The same technic led to the isolation of a strain of Leptospira from an assistant veterinarian who suffered from canicola fever.

Since it was rarely possible to study the dogs for any length of time, it is not unlikely that the negative findings on the urine of dogs to date are not due to faulty technic but, rather, are probably the result of inaccessibility to suitable material or are due to other conditions. It was impossible to correct the acid reaction of excreted urine by placing the dogs on an alkali-producing diet. Therefore, irrespective of the negative findings to date, urine specimens of recovered dogs should be examined both by animal inoculations and cultures.

IDENTIFICATION OF THE LEPTOSPIRA

Following our visit to the laboratory of Professor W. Schüffner in Amsterdam, five strains cultivated from dogs D62, D56, D64, D65, Santa Rosa, together with ten strains isolated from rats, were sent to him for examination. In his report he called attention to the important fact that the dog strains were serologically identical with *L. canicola*, while the rat strains were indistinguishable from *L. icterohemorrhagiae*, *S. icterogenes* or classical strain. The serological findings reported by B. Walch-Sorgdrager and W. Schüffner are shown in table II.

In 1934, Klarenbeek and Schüffner recognized the *L. canicola*, isolated for the first time in 1932, as a new species which differs serologically from the classical strains. Since then, the wide distribution of this species in Holland, Germany and Denmark as a causative agent of clinical and subclinical infections of dogs has been proved. Moreover, the independent position of the *L. canicola* has been fully documented by Walch-Sorgdrager and Schüffner, and others. Irrespective of the sweeping claims by Packchanian that this type is only a variant of the classical strain, the findings in California amply support the workers in Holland and warrant the naming of the new species *L. canicola*.

Through the courtesy of Professor W. Schüffner, type serums and Leptospira strains were made available. His observations have been fully confirmed through the examination of ten additional strains and through many direct serological tests employed in the diagnosis of this canine disease. The strains thus far isolated in San Francisco and throughout California from dogs are *L. canicola.*

TABLE II—Serological findings.

Strains	Antiserums of		
	L. Icterohemorrhagiae Titre 10,000	L. Canicola Titre 30,000	Rachmat (Sumatra Strain) Titre 30,000
Rat—219	30,000	10,000	300
Rat—City D	30,000	10,000	1,000
Rat—City F	30,000	3,000	0
Rat—La Salle	30,000	3,000	300
Rat—City C	30,000	3,000	3,000
Rat—509	30,000	1,000	300
Rat—221	10,000	1,000	100
Rat—City B	10,000	1,000	300
Rat—Original	10,000	300	0
Rat—City E	10,000	300	10
Dog—D64	1,000	30,000	0
Dog—D56	1,000	30,000	0
Dog—D65	300	30,000	0
Dog—Santa Rosa	300	30,000	0
Dog—D62	300	10,000	0

Serological Examinations

Serological methods are now extensively used not only to identify the different Leptospira strains but, more specifically, to diagnose the nature of an infection suspected of being Weil's disease. For ease, accuracy and speed these methods have not been surpassed. In fact, until the technic of these methods had been simplified, the extent of leptospirosis in a city or county remained largely unknown.

Agglutinins, lysins and complement-fixing antibodies appear towards the end of the first week of the disease, increase to reach a high level (1:300,000) within five to eight weeks, persist for many months and then decline within 1½ to 2½ years to a level of 1:300. Thus, retrospective diagnosis may be made many years after the attack. The action of the immune serum is controlled microscopically under darkfield illumination. When living Leptospirae are brought in contact with an active serum, both agglutination and lysis of the Leptospirae are observed. Highly active Leptospira serums may show a preëminently lytic action, while the immobilization, clumping and swelling of the Leptospira (agglutination) are nearly suppressed. We regard the lysis-agglutination phenomenon as being important in the serodiagnosis of leptospirosis. One of the observations made with one of the dog serums is illustrated in table III.

TABLE III—Observation on lysis agglutination.

Dilution	Agglutination	Lysis
Control	0	0
1:10	+++	0
1:30	+++	0
1:100	++	+
1:300	+	++
1:1,000	0	+++
1:3,000	0	+++
1:10,000	0	+++
1:30,000	0	++
1:100,000	0	0

When formalinized suspensions are used, agglutination is seen; in low dilution, slight lysis. The complement-fixation test according to Gaehtgens (1933) or the adhesion test of Brown requires a number of reagents without offering any advantages.

Pot and Smith and Tulloch have advocated the use of the macroscopic agglutination methods. In the comparatively few specimens studied by these procedures, the results have not proved as satisfactory, nor the tests as sensitive, as that which has been introduced by the Dutch worker. The technic of the lysin-agglutination test quite universally used is that described by Schüffner and Mochtar and reviewed by Davidson (1934). Living and formalinized (0.5 per cent highest purity neutral formalin, see Walch-Sorgdrager, Schüffner and Bohlander) antigens of four- to five-day-old heavy cultures of Leptospira in Verwoort's medium are used. As a diluent, the same

TABLE IV—Leptospira infections of dogs received at a hospital in San Francisco in 1939 (one serum from Detroit); titre of the seroreaction (agglutination).

DOG	CLASSICAL STRAIN	CANICOLA STRAIN	CLINICAL PICTURE
D118	(1) Negative (2) Negative (12 days later)	1:100++++; retest 1:10,000++++ (12 days later) 1:300,000++	Hemorrhagic; recovered
D120	Negative	1:30+++	Hemorrhagic; died
D122	(1) 1:10++++ (2) 1:100+++ (12 days later)	1:100++++; retest 1:1,000++++ 1:3,000++ (12 days later)	Hemorrhagic; recovered
D124	1:10++++; 1:30++	1:100++++; 1:300++	Hemorrhagic; died
D128	1:3,000++++	1:30+++; 1:100+	Icteric (serum from Detroit)
D129	1:10,000++++	1:100++	Icteric; died
D130	1:300++	1:10,000++++; 1:300,000+++	Hemorrhagic; died
D141	1:1,000++++	1:10,000++++; 1:300,000+++	Hemorrhagic; died
D143	1:10,000+++	1:30,000++++	Hemorrhagic; died
D144	1:100++++; 1:300++	1:1,000++++; 1:3,000++	Hemorrhagic; recovered
D145	1:300+; 1:100++; 1:30++++	1:3,000++; 1:1,000++++	Hemorrhagic; recovered Parshall, Petaluma
D112	1:3,000++++; 1:10,000+++	Negative	Icteric; recovered; history of eating rats
D147	1:3,000++++; 1:10,000+++	1:300++++; 1:3,000++	Hemorrhagic; died

medium without serum is employed. The technical details and methods of dilution are presented in appendix C.

For the routine examinations, the antigens of *L. icterohemorrhagiae* (classical strain) and *L. canicola* have been used. Since these two types are serologically distinct, it is reasonable to suspect that other antigenic Leptospira variants may in time be encountered and additional strains may have to be included in the tests.

According to Walch-Sorgdrager and Bohlander, a study of 300 cultures with monovalent rabbit anti-serums disclosed 11 to 16 groups of Leptospirae. The basic observations by Walch-Sorgdrager and Schüffner that the simple agglutination and, less frequently, that the lysin tests furnish dependable and reproducible results have been amply confirmed by studies in California. Until further antigen analyses have been made and more strains isolated from the dogs, the local tests are best limited to the two types. In this connection, it is recalled that Esseveld isolated *L. hebdomadis* from the dog in Java and the Australian type "Ballico" from Celebes. Thus, in other countries dependent on the virus reservoirs many other strains may have to be included as antigens in the tests.

It appears unnecessary to detail the reactions which have been secured in a series of several hundred tests but in order to illustrate the agglutination titers, a few of the more recent examinations on specimens submitted for diagnosis are listed in table IV.

With the aid of these tests, the nature of Leptospira disease in the two different clinical-anatomical types has been conclusively proved, but with the same procedure the existence of a great many latent infections has been recognized.

As a rule, the titre of the reactions with the two antigens is sufficiently striking to enable the examiner to render a definite diagnosis relative to the type of Leptospira which caused the infection. Strongly positive coreactions, as, for example, noted with serum D143 in table IV, may require absorption tests or, preferably, if in any way practical, repeated examinations at intervals of one to two weeks or the isolation of the Leptospira.

For the identification of the freshly isolated Leptospira strains, cross agglutination and absorption tests with known positive rabbit serums have proved quite reliable. At least, the studies in connection with canine leptospirosis in California support in every respect the observations made in Holland. Therefore, only monovalent serums have been employed. The nonspecific coreaction of the respective serums has always been of a much lower titre and a decision relative to the nature of the infections has, as a rule, offered no diffi-

culties. In consequence, it has not been necessary to resort to highly specific polyvalent antiserums, as recommended by Schlossberger and Pohlmann.

It is noteworthy that, to date, *L. icterohemorrhagiae* or the classical strain has not been isolated from canine infections. Quite recently, the serums of two icteric and one hemorrhagic dogs (Los Angeles) gave specific reactions with this strain. According to the history, at least one of the dogs infected with the classical strain was observed in the act of eating rats. The significance of these findings will be considered in connection with the epizoötiology and the latent infections.

Experimental Disease

The difficulties encountered in attempts to use the guinea pig as an experimental animal to reproduce a clinically or anatomically typical leptospirosis have already been recorded. Inoculations with pure cultures clearly proved the low pathogenicity of the majority of California *L. canicola* for these rodents. The data concerning the findings on guinea pigs inoculated with organ suspensions of dogs, which yielded positive Leptospira blood cultures, are summarized in table V. There is also included for comparison the findings secured with a specimen of human urine containing *L. canicola*.

The kidneys of five dogs apparently contained sufficient Leptospirae to infect guinea pigs in the first or second passage. Fatal infections were produced by the strains of three dogs. The guinea pigs died on the 9th to 15th day with positive Leptospira findings in the liver and peritoneal fluid. Icterus was present only in two guinea pigs injected with the urine of a dog. On second passage two other strains caused fatal infections. The lethality was increased from 54 to 84 per cent. One strain (D72) repeatedly through the third and fourth passage produced jaundice. The strain of *L. canicola* of human origin likewise became slightly more pathogenic; icteric discoloration was noted in the animals. Provided the organs and blood of the guinea pigs were not contaminated with bacteria, Leptospira cultures were secured from the liver and peritoneal fluid. The pathogenicity of the 15 pure cultures of *L. canicola* was tested on young guinea pigs, with the results shown in table VI.

Tested shortly after isolation, seven (46 per cent) of the 15 strains induced fatal infection. The four strains retained their pathogenicity through several passages while three lost it. Two strains (D95 and D96) in the third passage and one in the fourth caused lethal infections. The number of animals used is not large, but it must be recognized that the *L. canicola* may kill guinea pigs following the primary inoculation (Santa Rosa and D97) and retain this property for some time. On the other hand, the majority of strains possess a low infectiousness. In this respect, the California canicola cultures differ somewhat from those isolated in Holland. Equally important is the fact that icterus was constantly seen in the guinea pigs injected with one strain (D72).

The canicola strain of human origin gradually acquired the property to induce jaundice. Despite the increased infectiousness, the tendency to icterus failed to become enhanced. In this respect, the canicola types differ fundamentally from the classical icterohemorrhagiae strains, which become more or less capable of causing liver damage as the pathogenicity rises or falls.

The observations in California amply confirm the findings of Walch-Sorgdrager and Schüffner that the icterogenic tendencies of *L. canicola* are a fixed property of certain strains. Since the isolation of the Leptospira was the primary purpose of the passage experiments, few guinea pigs were held long enough for us to test their urine for the presence of organisms. In the few instances in which examinations were made, however, Leptospirae were not demonstrable. In conjunction with the similar negative findings in dogs, it appears not unlikely that a leptospiruria is not a very common sequel to a canicola infection. On the other hand, it should be noted that the *L. canicola* of human origin was obtained from the urine of the patient. Therefore, before a final word is spoken, additional observations should be made.

Common to the *L. canicola* strain is the

TABLE V—Injection of dog material into guinea pigs (dogs from which cultures were isolated).

Material from dog	Passage 1				Passage 2				Passage 3				Passage 4				Passage 5				
	Number of pigs	Leptospira	Intersus	Death	Number of pigs	Leptospira	Intersus	Death	Number of pigs	Leptospira	Intersus	Death	Number of pigs	Leptospira	Intersus	Death	Number of pigs	Leptospira	Intersus	Death	
Santa Rosa (urine)	2	Liver	+	D 11 days	Not carried further																
Dog 56	No organs into pigs																				
Dog 62 (blood)	2	0	0	K 11 days	2	0	0	K 11 days	1	0	0	K 11 days	1	0	0	K 11 days	2	0	0	K 11 days	Discard
Dog 64 (organs)	2	0	0	D 1 day	Highly contaminated																
Dog 65 (kidney)	2	0	0	K 11 days	2	0	0	K 11 days	2	0	0	K 11 days	1	0	0	K 14 days	Discarded Blood cultures O.K.				
Dog 78 (kidney)	2	0	0	K 11 days	2	Cultures contam.	+	D 11 days	2	Perit. fluid Liver	+	K 7 days	1	Liver	+	K 6 days					
Dog 90 (blood clot in saline)	2	0	0	K 11 days	1	Cultures negative	Sbc hem.	K (ill) 6 days	2	0	0	K 9 days	1	0	Sbc hem.	K 11 days	1	0	0	K 11 days	Discard
Dog 94 (kidney)	2	Contam. cultures	+ Mild	K 14 days	1	Cultures negative	Hem.	K (ill) 6 days	1	Cultures negative	0	D 14 days	1	0	Hem. lungs	K 11 days	1	0	0	K 11 days	Discard
Dog 95 (blood)	1	0	Hem. lungs	K 14 days	1	Liver	Sbc hem.	D 8 days	1	Cultures contam.	Sbc hem.	D 8 days	1	Liver	Sbc hem.	D 7 days					
Dog 96 (blood)	1	0	0	K 11 days	1	Liver	0	K (ill) 9 days	1	Liver	0	D 7 days	Not carried further								
Dog 97 (kidney)	2	Perit. fluid Liver	Sbc hem.	D 9 days	1	Perit. fluid Liver Heart	Sbc hem.	D 7 days													
Dog 110 (kidney)	1	0	0	K 12 days	1	0	0	K 11 days	No third passage - not carried further - cultures of blood O.K. (no pigs)												
Dog 113 (organs)	2	0	0	K 9 days	2	0	0	K 11 days	Not carried further - cultures of blood O.K. (no pigs)												
Dog 115 (kidney)	2	Liver	Sbc hem. Hem. lungs	D 15 days	1	Liver Heart	Sbc hem. Hem. lungs	D 14 days	Not carried further												
Morensoni (urine 29th day) (Human)	2	0	0	K 12 days	2	Liver Spleen	+ (Mild)	D 13 days	1	Heart Liver	+	D 6 days									

low infectiousness for other rodents. Experiments with deer mice have already been mentioned. Suffice it to note that neither wild nor norvegicus rats nor white mice proved susceptible to an infection with the California strains. These laboratory tests merely support the epizoötiological investigations made both in Holland and in San Francisco which conclusively proved the absence of canicola strains in the rat population. Young rabbits also proved quite resistant, but repeated injections of formalinized four-day-old cultures stimulated the production of agglutinins in high titres.

The pathogenicity tests strongly indicated that the Leptospirae isolated from the dogs in San Francisco were never transmitted to rodents but maintained their infection chain in the canine population. Experiments were consequently undertaken to reproduce the malady in dogs. Young (six months to one year) and old (two to six years) dogs of various breeds were injected intraperitoneally, intramuscularly and intravenously with infected organ suspensions, and cultures of recently isolated Leptospira strains. Equally by feeding and by nasal or throat sprays, the infectious material was applied.

These tests, limited to ten days, were successful only in part. The dogs inoculated showed no loss in weight, no rise in temperature or positive Leptospira findings in the urine or at autopsy in the sections of the liver and kidneys. At the time these experiments were in progress, a dependable serological test had not been developed. Subsequently, with a reliable procedure at hand, the serums of some of the experimentally infected dogs were tested and found to have titres varying from 1:300 to 1:600. Thus, only subclinical infections of the nature quite frequently encountered in the dogs at large in any community were experimentally induced. Additional tests on a larger series of dogs should be made

with freshly isolated strains of Leptospira.

Further studies in the direction to reproduce clinically typical canicola leptospirosis in the dog should be undertaken on a series of animals preferably secured from an area in which the malady has not been observed in enzoötic distribution. In this connection, a significant statement by Klarenbeek (Congress, p. 350) deserves consideration: "Although one may anticipate," he says, "a characteristic course of the infection following the intravenous or intraperitoneal injection of Leptospira, certain symptoms may not always be noticeable; in fact, many may be missing." In other words, the experimental reproduction of canine leptospirosis may offer considerable difficulties. However, it should be noted that in another paragraph he reports on the ready transfer of the Leptospira to a dog by intravaginal insertion of a cotton plug soaked with urine of a dog suffering from leptospiruria.

Wirth emphasizes that young dogs should be chosen for the experimental infections with *L. canicola*. By following this principle, he apparently succeeded in producing fatal infections in puppies. The animals died on the 17th to 18th day after the injection of the cultures. The records of the experiments are not given in detail, and it is not entirely clear whether the classical or canicola strains were used for the tests. The early experiments of Uhlenhuth and Fromme, Krukenberg, Nicolle and Lebailly (1918) to transmit *L. icterohemorrhagiae* were unsuccessful. On the other hand, Courmont and Durand (1917), Monti, Okell, Dalling and Pugh (1924) report on fatal infections in young dogs with typical lesions following intravenous or intraperitoneal injections and even feeding of infectious material.

Since both young and old dogs contract canicola leptospirosis spontaneously and these ages have been included in the infection experiments, it is probably correct to conclude that the susceptibility of the

TABLE VI—Guinea pig inoculations with pure cultures of Leptospira.

Strain	Passage 1				Passage 2				Passage 3				Passage 4				Passage 5			
	Number of pigs	Lepto-spira	Icterus	Death	Number of pigs	Lepto-spira	Icterus	Death	Number of pigs	Lepto-spira	Icterus	Death	Number of pigs	Lepto-spira	Icterus	Death	Number of pigs	Lepto-spira	Icterus	Death
Santa Rosa (0.5 cc. i.p.)	2	Liver	0	D 11 days	2	Liver Heart	0	D 11 days	(Tested shortly after isolation)											
D 56 (0.5 cc. i.p.)	2	0	0	K 11 days	1	0	0	K 11 days	1	0	0	K 11 days	1	0	0	K 11 days	(December 1938 - not carried further)			
	1	Liver	0	D 15 days	1	Liver Heart	0	D 10 days	(Shortly after isolation)											
D 62 (0.5 cc. i.p.)	2	Cultures negative	Sbc hem.	D 20 days	1	0	0	K 11 days	1	0	0	K 11 days	1	Liver	Sbc hem. Lungs hem.	D 11 days	(Shortly after isolation)			
D 64 (0.5 cc. i.p.)	2	0	0	K 11 days	2	0	0	K 11 days	1	0	0	K 11 days	1	0	0	K 11 days	1	.0	0	K 11 days
																	(December 1938 - not carried further)			
D 65 (0.5 cc. i.p.)	2	0	0	K 10 days	2	0	0	K 10 days	1	0	0	K 10 days	2	0	0	K 11 days	2	0	0	K 11 days
																	(December 1938 - not carried further)			
D 78 (0.5 cc. i.p.)	2	Liver Heart	+	D 10 days	2	Liver Heart Perit. fluid	+	D 9 days	(December 1938)											
D 90 (0.5 cc. i.p.)	2	0	0	K 11 days	2	0	0	K 11 days	2	0	Sbc hem.	K 11 days	2	Liver (1)	Lung hem.	K 11 days	1	Liver	Sbc Lung hem.	K 11 days
																	(Shortly after isolation)			
D 94 (0.5 cc. i.p.)	2	0	0	K 11 days	2	0	Lung hem.	K 11 days	2	0	Lung hem.	K 11 days	1	Liver	Lung Sbc hem.	K 11 days	1	Liver	Lung Sbc hem.	D 11 days
																	(Shortly after isolation)			
D 95 (0.5 cc. i.p.)	1	0	0	K 11 days	1	0	Lung hem.	K 11 days	1	Liver	Lung hem.	D 10 days	(Not carried further) (Shortly after isolation)							
D 96 (0.5 cc. i.p.)	1	0	0	K 11 days	1	0	Sbc hem.	K 11 days	1	Liver	Sbc hem.	D 11 days	(Shortly after isolation)							
D 97 (0.5 cc. i.p.)	1	Liver	Sbc hem.	D 10 days	(After isolation) (Not carried further)															
D 110 (0.5 cc. i.p.)	1	Liver Heart	Sbc hem.	D 10 days	1	Liver Heart	+	D 10 days	(Shortly after isolation)											
D 113 (0.5 cc. i.p.)	1	Liver	Sbc hem.	D 10 days	(Not carried further - shortly after isolation)															
D 118 (0.5 cc. i.p.)	1	0	0	K 11 days	1	0	Hem. sbc	K 11 days	1	Liver Heart	Sbc hem. Lung hem.	D 12 days	(Shortly after isolation)							
Morenami (0.5 cc. i.p.)	2	0	0	K 11 days	2	0	Lung hem.	K 11 days	1	Liver	+ Slight	D 10 days	(Shortly after isolation)							

canine species to the infection, although high, is quite low as far as the disease is concerned.

DIAGNOSIS

As the observations on canine leptospirosis progressed, certain procedures adapted to the diagnosis of these infections were developed. Direct and indirect methods in the following order are now used: 1) Lysin-agglutination test; 2) blood cultures and guinea pig inoculations with blood; and 3) urine examination by darkfield, serological procedures, cultures and animal tests.

1) By far the most convincing evidence that leptospirosis exists in the dog population of San Francisco was secured with the serological tests. As already pointed out, the hemorrhagic type of the disease rarely lends itself to cultural studies. Recent findings, however, indicate that significant serum reactions may be recorded in the early stages of the disease, and thus a correct differential diagnosis from a severe gastrointestinal disease or blacktongue may be recorded with ease. Furthermore, the serodiagnosis offers other advantages. The results of the tests are available within a few hours; they may be repeated in the course of the malady; they recognize the type of leptospiral infection and, since the antibodies persist for many months, anamnestic diagnoses may be rendered. Finally, the serum examinations furnish invaluable information relative to the extent of latent infections in the dog population. In the hands of the experienced, the methods have proved reliable and readily reproducible, provided sterile serums, which are not hemolyzed, are furnished to the laboratory at any stage of the malady.

2) Contrary to published statements, a darkfield examination of the peripheral blood of a dog suffering from clinical leptospirosis at the same time the animal is brought to a veterinarian rarely reveals the organisms. Moreover, the type of examination is fraught with many pitfalls, as has already been pointed out. However, cultures prepared from the defibrinated blood or clot in Schüffner media appear, in the light of the California and Dutch experiences, to be the most promising procedure to isolate the Leptospira responsible for the disease in a pure state. Of the 14 cultures secured from sick dogs, twelve were isolated in direct blood cultures, which were made in nine instances with blood collected two days to a few hours before deaths. Two cultures were taken from dogs in the advanced stages of the disease. The animals were deeply jaundiced and in a state of collapse. These dogs eventually recovered. One culture was isolated from clotted blood which had been removed from the heart shortly after death. The impression quite generally prevails that a search for Leptospirae has little practical value when the animals are jaundiced and the illness is already of one-week duration. Experience has taught that the defibrinated blood is in all likelihood the best source to secure a pure culture of the Leptospira in the Verwoort media. Passage of blood specimens through guinea pigs may be equally successful. However, repeated transfers may be required and, thus, ultimate isolation of the Leptospira may be greatly delayed.

3) The examination of the urine by various methods has under the present existing conditions not proved very practical, since suitable samples are rarely obtainable. Centrifuged specimens may be examined in the darkfield or may be cultured in the Verwoort fluid if secured by catheter and free from bacteria. They also may be inoculated into the guinea pig. However, it is important to change the reaction of the urine to a pH above 7.0 by keeping the dogs on an alkali-producing diet. Samples of urine with a high albumin content should be repeatedly examined for Leptospira, since the number of the organism is subject to great variations, dependent on the discharge from the tubuli. In fact, from a public health standpoint, it is imperative that no dog should be returned to its owner without such an examination.

The risk that a dog with leptospiruria may infect man has been amply proved in California, Holland and Denmark. A urine examination has, therefore, not only a diagnostic but also a preventive aspect. Unfortunately, the present trend in small animal practice rarely lends itself to careful

studies of the urine and, consequently, little is known relative to the Leptospira shedder stage, which may follow clinical or subclinical infections.

Van der Hoeden noted the presence of specific antibodies in the urine specimens obtained from cases of leptospirosis. In a series of 18 cases examined, 17 showed agglutinins in the urine. Antibodies appeared in the secretion a few days after they had been demonstrated in the blood stream. Only two California dogs were tested by this method. In one dog with a serum-agglutination titre of 1:10,000, the urine lysed and clumped from the same antigen in a 1:10 dilution. The second, with an identical serum titre, agglutinated the antigen with a 1:30 dilution of the urine.

Since the antibodies persist for many months, it is apparent that the serodiagnosis on urine specimens may be used for epizoötiological studies. Although the agglutinins only appear following an infection, it is nevertheless advisable not to replace the direct cultural studies by this indirect procedure. Finally, it should be remembered that the titre of the agglutinins or lysins is frequently quite low; in consequence, the dilutions should begin ½, ¼, ⅛, etc.

EPIZOÖTIOLOGY

The following facts concerning the epizoötiology deserve brief consideration. In San Francisco and elsewhere in California, clinical leptospirosis affects more frequently dogs 3 to 8 years of age. Only four of the 120 dogs observed were 5 to 10 months old. Canines of all breeds have been found susceptible to *L. canicola* infection. Of the 101 dogs included in the series that we observed, 79 were males and 22 females. The highest incidence of leptospirosis in the vicinity of San Francisco occurs during May and July and again in November and December, as is indicated by the data in the following tabulation covering autopsies, clinical cases and diagnoses based on serological tests collected during the past two years.

Cases of Canine Leptospirosis, According to Months, in San Francisco

January	3
February	7
March	12
April	11
May	15
June	6
July	18
August	16
September	6
October	5
November	11
December	10
	120

Too much weight should not be given to these statistics, since the dogs were received from one or two sources and, then, only when it was convenient to deliver the animals to the laboratory. According to reliable information, leptospirosis in other sections of California has, however, a similar distribution of incidence and sex, as indicated by the data here presented. In Holland, the highest incidence occurs during the months of October and November. In California the predominance of clinical leptospirosis in males (79 males : 22 females) is significant for various reasons. It is quite generally known that leptospiral infections are more frequently seen in male dogs. For example, in Holland the malady due to the classical strains shows a relationship of three males to two females, while the canicola infections are decidedly more common in males (10 : 2). These differences are ultimately connected with the infection chain.

L. canicola maintains its host to host relationship through general uncleanliness by simple contact, by being eliminated in the urine of clinical cases or convalescent and chronic carriers. Dogs are accustomed to licking urine or coming intimately in contact with the genitalia. No distorted pathways of infection are necessary; the infective agent enters the body on the tongue or nose. Since these habits are more common for the male than for the female dog, a higher incidence of canicola fever in the former is readily explainable. On the other hand, it is not unlikely that the sexual act may convey the infection. Canine leptospirosis due to the classical strains probably depends on sources other than the mere

passage of the organism from dog to dog. The eating of rat carcasses and the drinking of water or food soiled with urine from murine shedders of spirochetes are equally important. Thus, female dogs may have an equal chance of becoming exposed and, although the infection is still slightly higher in males, the proportion males : females in Holland is changed from 10 : 2 to 3 : 2. Whether the same relationship holds true in the United States requires further inquiry.

Due to the fact that studies here reported have been made principally in San Francisco, where rat proofing has been developed to a high level, only three classical cases against 33 of canicola fever have been identified. In Holland, the proportion of classical to canicola in percentage is 38 to 62. Dog serums submitted from Detroit, Mich., yielded reactions which would indicate only classical infections (ten of twelve serums react with the classical strains).

Unfortunately, the available records give little accurate information relative to the morbidity and the case mortality. At the hospitals, many of the dogs were destroyed for humanitarian reasons while others considered doomed were nursed to recovery at the laboratory. It was the high mortality of 80 per cent at the dog hospital which prompted Dr. Michael to ask for assistance. This figure is probably too high. According to Klarenbeek in Holland, the case mortality is 51 per cent for classical and 41 per cent for canicola infection. By comparison, even this rate requires correction, since the many atypical, abortive and latent infections have not been taken into consideration.

LATENT INFECTIONS

In order to judge the incidence of canine leptospirosis, in particular the extent of the canicola infection in northern California, series of serums were subjected to serological tests. The animals were clinically well while they were bled. Of 47 dogs collected from the vicinity of San Francisco and the rural area of northern California, 16, or 34.04 per cent, showed definite evidences of contact with *L. canicola*. The titre varied from 1:100 to 1:300,000 with coreactions of *L. icterohemorrhagiae* 1:10 to 1:1,000.

In a group of 28 dogs in Santa Rosa only 4, or 14.3 per cent, gave similar reactions. Two of the animals with positive serological findings had a history of illness with jaundice. Thus, the random sampling of 75 dogs in northern California disclosed the important fact that nearly one-fourth of the animals had passed through an attack of subclinical leptospirosis. For comparison, 111 serums, secured from the vicinity of New York City through the courtesy of Norman J. Pyle of the Lederle Laboratories, comprising 13 different breeds ranging in age from 1 month to 12 years, were subjected to serological tests. Ten dogs (9 per cent) agglutinated *L. canicola* in dilutions of 1:100 to 1:300,000, while 3, or 2.7 per cent, reacted with *L. icterohemorrhagiae*.

Through the observations of Jungherr, canine leptospirosis had been suspected in the Atlantic States, but for the first time it is now proved that both the classical and the canicola spirochetes may cause clinical and latent infections. The limited number of dog serums (21) obtained through the courtesy of Charles N. Leach of the Rockefeller Rabies Station at Kilby, near Montgomery, Ala., and collected from dogs of the rural districts of the South failed to yield reactions indicative of latent infections. As already mentioned, the few samples from Detroit were submitted because the dogs were in some way connected with cases of human Weil's disease.

The exclusive findings of classical infections do not signify that this type is the only Leptospira which exists in the dog population. As a rule, in dog serums collected at random, canicola overshadows the classical infections. This is well illustrated by the data collected from the public records and shown in table VII.

A scrutiny of the tabulated observation reveals that from 2 to 46 per cent—on the average, one-quarter of the dog population of different countries sampled at a given time—show the residuals of a Leptospira infection. The clinical cases are merely

accidents incidental to a vast, latent epizoötic constantly maintained by subclinical infections leading to a reservoir of carriers. The magnitude of this reservoir is yet unknown but since the statistical data of Holland correspond in general with those collected for the United States, probably between 20 and 50 per cent temporary or permanent Leptospira carriers and shedders may be anticipated.

TABLE VII—Clinical and latent canine leptospirosis.

Titre of Serum >1:100	Holland			Germany		Denmark	United States				
							Calif.		N. Y.	Mich.	Ala.
	Clinical Cases*	Latent at Random†	Latent at Clinic‡	Latent at Random§	Latent ‖	One Village	Clinical	Latent	Latent	Latent	Latent
L. icterohemorrhagiae	57 (38%)	10 (13%)	4 (8%)	3 (6%)	1	18 (34%)	3 (5%)	0	3 (2.7%)	10	0
L. canicola (mixed)	94 (62%)	25 (32%)	10 (20%)	6 (12%)	3 (1.5%)	1	33 (55%)	20 (26%)	10 (9%)	0	0
Total No. dogs examined	151	76	50	48	200	53	59	75	111	13	21

*Reported by Klarenbeek and Winsser, 1938.
†Klarenbeek and Winsser, 1938.
‡D. J. Kok, 1938.
§Uhlenhuth and Zimmerman, 1936.
‖Dahr, 1937.

Although the data for the United States are not sufficiently detailed to draw definite conclusions, it is evident that the incidence of latent infections increases with age. Kok in Holland found nearly 50 per cent of the dogs over 6 years of age to show the residuals of latent infections.

Leptospirosis of Rats

At the beginning of the investigation and before the causative agent had been serologically identified, the association of Leptospira with the canine disease suggested some epizoötiological relationship with rats. Although murine leptospirosis had been previously recognized by J. R. Ridlow, it appeared of interest to determine the extent of the infection and to study the organisms. Random sampling of the kidneys of 467 rats (*M. alexandrinus, M. norvegicus* and *M. rattus*) revealed in the darkfield and silver-stained methods that 76, or 35 per cent, were infected with Leptospira. Through guinea pig passage, 23 strains were isolated and serologically identified, with the assistance of Professor W. Schüffner, as *L. icterohemorrhagiae*. All the strains of murine origin were highly infectious for guinea pigs, causing fatal infections with icterus and general hemorrhages on the first, second or third passage. The *L. canicola* has not been encountered in the rat population. Based on a much larger series of rat strains collected over a period of four years, Walch-Sorgdrager and Schüffner have both failed to isolate the *L. canicola* from this rodent. The rats had been caught in cities in which canine leptospirosis is quite extensive, and doubtless ample opportunity was afforded the rats to come in contact with the disease agent. One is unquestionably justified to conclude that the rat is not a natural host for *L. canicola*, and that this relationship of the *L. icterohemorrhagiae* to rats and of the *L. canicola* to dogs is equally true for California.

Public Health Significance of Canine Leptospirosis

As early as 1933, Klarenbeek and associates suggested the possibility of aberrant human infections with the *L. canicola*. At least 21 of these infections have been recognized as follows: Twelve in Holland (Walch-Sorgdrager and Schüffner), one in Austria (Wirth), six in Denmark (Brammer, Petersen and Scheel-Thomsen) and two in California (Meyer, Stewart-Anderson and Eddie).

How often this infection occurs is not known. Canicola fever of man is difficult to diagnose clinically, since the majority of cases have no jaundice, resemble influenza, or run an undulant fever-like course frequently associated with meningitis. Only in one of the California cases was a definite icterus present; the second case had an influenza-like attack with temperatures varying from 103° to 106° F., severe headache and nausea, although he was ambulatory. Without a serological examination of his blood, which suggested itself in view of the history that he acted as assistant in a dog pound, the nature of his infection would not have been recognized. He was ill with frequent remission of febrile bouts for at least one month. On the 36th day of his sickness, *L. canicola* was isolated from his urine through guinea pig passage.

Mild cases of canicola fever are difficult to recognize without a bacteriological examination. Veterinarians constantly exposed to dogs should remember that canicola fever is an occupational hazard. Precautions should be taken to prevent the return of dogs recovered from jaundice or typhus to their owners without extending a warning that uncleanliness and, particularly, the handling of urine exposes them or other members of the household to the risk of canicola fever. In order to reduce the likelihood to a minimum, it is recommended that dogs with positive urinary findings of Leptospira be placed on an exclusive meat diet or given hydrochloric acid in drinking water. Such treatment renders the urine strongly acid and, therefore, unsuitable for the Leptospira. These principles apply equally well to the Weil's disease of dogs caused by classical strains. Human infections caused by this type are more serious, since a mortality of 10 to 25 per cent has been reported.

SUMMARY AND DISCUSSION

The existence of canine leptospirosis in California and in certain sections of the United States has been conclusively established by bacteriological and serological examination and animal inoculation. The first isolation of *Leptospira canicola*—one of the causative agents of this disease—was made in 1937 and reported by Meyer and coworkers in 1938. The classical strain, *L. icterohemorrhagiae*, has not as yet been cultured from dogs, but its existence as a factor in certain clinical as well as latent infections is amply proved by serological findings made on the serums of dogs in New York City and Detroit, Mich. The epizoötiology of the disease is remarkably similar to that established by Klarenbeek, Schüffner and others for Holland.

Canicola infections are confined to dogs and the infections chain maintains itself through simple contact. The urine of the recovered animals, which is frequently heavily charged with the Leptospira growing in the tubules of the kidneys, serves, as far as is known, as the principal source of infection. Until quite recently, the *L. canicola* was the only type of Leptospira known to be associated with two clinically different types of disease.

The malady associated with icterus resembles in every respect canine Weil's disease reported from England, Holland, Germany and Austria, which is in 80 per cent of the cases induced by the classical strain. In fact, the European observers emphasize the extreme rarity of jaundice in pure canicola fever. Klarenbeek, in 94 spontaneous canicola infections, noted icterus in only three. More recently, however, in collaboration with Winsser, he reported that the experimental subcutaneous infection in pups produced icterus in almost all cases.

Equally intriguing is another difference. The so-called hemorrhagic disease in California, very rarely accompanied by a faint icterus, clinically and anatomically indistinguishable from dog typhus, is, at least in California, definitely caused by *L. canicola*. A careful perusal of the literature of Holland reveals no record of this form of canicola fever, although Schlossberger and others have brought forth convincing serological evidence that the *L. icterohemorrhagiae* produces this clinical picture in dogs. Thus, in California, *L. canicola* induces in dogs two clinical entities which, as a rule, are quite rare in Europe. The exact nature of the hemorrhagic disease

was not recognized until serological methods and the examination of the kidneys disclosed the presence of the Leptospira.

Progress has been greatly delayed on account of the unfortunate tenacious adherence to the belief that every case of canine blacktongue is due to a dietary vitamin G deficiency, as originally established by Goldberger for the dogs in the rural areas of the southern states. It is at least significant to note a very low incidence of canine leptospirosis in the pellagrin areas, but there are increasingly frequent reports of so-called blacktongue in cities and towns in which the dog population is rarely forced to live on a deficient diet.

The Dutch veterinarians have observed a form of chronic leptospirosis predominantly characterized by a nephritis, high nonprotein-nitrogen content of the blood and uremia. Thus far, this type has not been noted in San Francisco but there is every reason to believe that it occurs. Systematic examinations of the urine for Leptospira, particularly in dogs with positive serum findings for these organisms, will readily disclose their existence. It would be of interest to know the incidence of this complication, since canicola fever in Europe is known to cause quite often uremia and leptospiruria (59 per cent). In the few cases available for a study in California, a nephritis, as evidenced by the high albumin content of the urine specimens, was in existence but Leptospirae were not demonstrable. The epizoötic distributions among the dog population could not be maintained without a high percentage of carriers and active shedders of the Leptospira.

Finally, it must be emphasized that many atypical and abortive illnesses may be subclinical or latent leptospirosis. These unrecognized and unidentified infections again leave in their wake a horde of shedders capable of spreading insidiously the Leptospira to other dogs and to man. The signs and symptoms in the latter host are so variable and the course often so mild that the infection remains unrecognized.

The diagnostic methods detailed in this paper deserve the widest application by workers familiar with their pitfalls and technical difficulties.

The treatment of Leptospira infections consists of the administration of specific antiserums. Either convalescent serum or serums of rabbits and horses immunized against the specific strains have been used in California. If administered during the first ten days of the disease, the immediate effect has frequently been striking, particularly when large doses (10 to 50 cc.) of serum have been injected intravenously. Since nearly 90 per cent of the cases of canine leptospirosis are caused by *L. canicola*, monovalent serums of a high agglutination titre may be used without delay. In regions in which both types of Leptospira are involved, polyvalent serums should be available, since no time should be wasted in trying to determine the nature of the strain responsible for the disease. There is no specific chemotherapy with arsenicals. In fact, drugs should be used sparingly. Intravenous administration of glucose solutions are beneficial and the loss of weight may be controlled by a liquid, high-calorie, carbohydrate diet.

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Appendix A

Silver Impregnation Method for the Demonstration of Leptospira

1) Blocs of tissues not exceeding 1 cm. in diameter are fixed in 10 per cent formalin in distilled water for several days or weeks, preferably three months.

2) Harden in absolute alcohol for 24 to 48 hours.

3) Pass through alcohol (70 per cent, 50 per cent, 30 per cent) to distilled water until the tissues are freed from alcohol.

4) Immerse in a 0.25 to 0.5 per cent silver nitrate solution in distilled water for 18 to 24 hours at 37° C. in the dark.

5) Wash thoroughly in distilled water. Change the water several times.

6) Place in 0.5 to 1 per cent hydroquinone in 50 per cent alcohol for 18 to 24 hours at room temperature.

7) Wash in 70 per cent alcohol; dehydrate in 95 per cent and absolute alcohol; clear in xylol; embed in paraffin.

8) Prepare very thin sections (3-4 $\mu\mu$). Remove paraffin and mount in balsam.

Appendix B

Schüffner Modification of Verwoort's Medium

Use 1.5 liters tap water (free from chlorine; rain water may be used instead of distilled water) and 1.5 Gm. Witte peptone. Boil.

Add 6 cc. phosphate mixture composed of 0.35 Gm. potassium phosphate (monobasic), 1.33 Gm. sodium phosphate (dibasic) and 100 cc. distilled water.

Boil. Add 300 cc. Ringer's solution composed of 0.8 Gm. sodium chloride, 0.02 Gm. calcium chloride, 0.02 Gm. potassium chloride, 0.02 Gm. sodium bicarbonate and 100 cc. distilled water.

Continue boiling. Add 150 cc. Sorenson's buffer solution—pH 7.2. Boil until precipitation is complete (about 30 minutes). Cool in ice box overnight. Filter. Test pH, which should be 6.8-7.2. Bottle. Autoclave at 15 pounds for 15 minutes. The medium may be kept in this state in capped bottles until needed for use. For use add 8-10 per cent sterile rabbit serum, tube in 2.5- to 3-cc. amounts, and inactivate for 30 minutes in a 56° C. water bath.

Test for sterility. Transplant, using a 1-cc. pipette.

Appendix C

Leptospira Agglutination Test Porcelain-Plate Method

Antigen.—Use a 4- to 6-day-old culture of

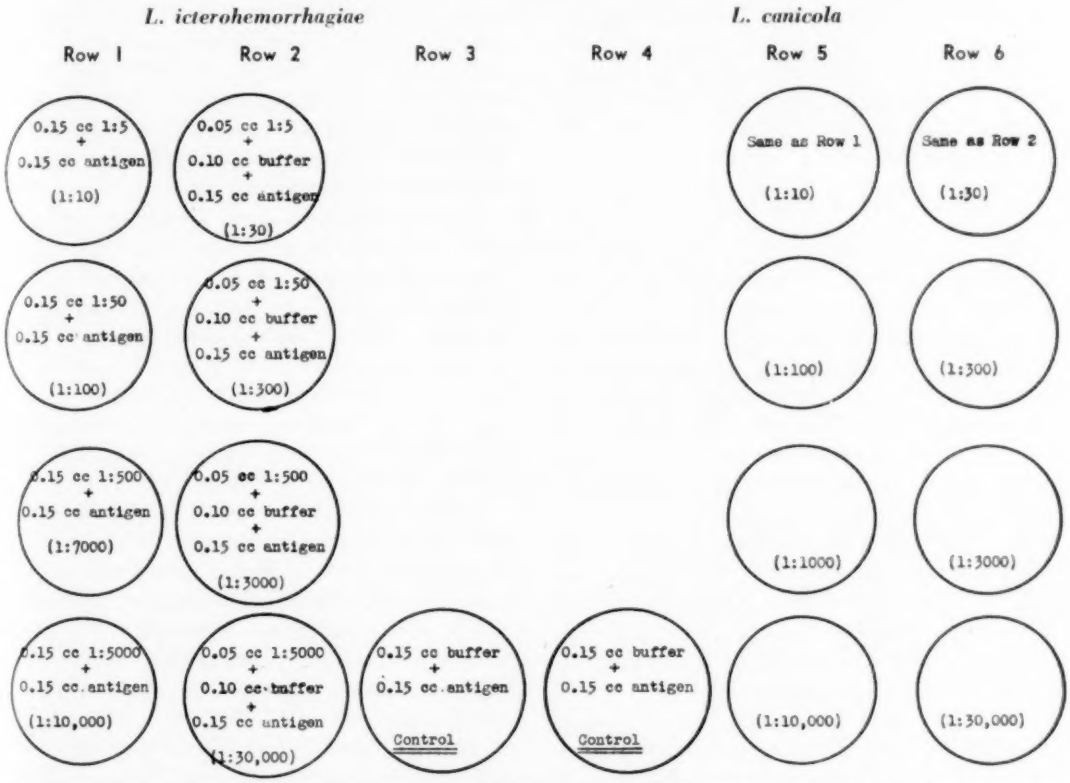
each strain. Use both live and formalin-killed antigen (three drops commercial formalin per 10 cc. culture). Incubate plates containing live antigen for two hours at 37° C. and examine each dilution by darkfield for lysis. Incubate plates containing formalin-killed antigen for four hours at 37° C. Examine each dilution by darkfield for agglutination or clumping.

Test.—For each serum specimen place four test tubes in rack. Use Verwoort-Schüffner buffer solution without serum as diluent.

Tube	1	2	3	4
Buffer	1.2 cc.	0.9 cc.	0.9 cc.	0.9 cc.
Serum*	0.3 cc.	0.1 cc.	0.1 cc.	0.1 cc.
Dilution	1:5	1:50	1:500	1:5,000

Use two porcelain plates for each specimen, one for live antigen, one for formalinized killed antigen. Set up both as in model below.

*Transfer 0.1 cc. from 2 to 3 and same amount from 3 to 4.


L. icterohemorrhagiae
L. canicola
Row 1
Row 2
Row 3
Row 4
Row 5
Row 6
0.15 cc 1:5 + 0.15 cc antigen (1:10)
0.05 cc 1:5 + 0.10 cc buffer + 0.15 cc antigen (1:30)
Same as Row 1 (1:10)
Same as Row 2 (1:30)
0.15 cc 1:50 + 0.15 cc antigen (1:100)
0.05 cc 1:50 + 0.10 cc buffer + 0.15 cc antigen (1:300)
(1:100)
(1:300)
0.15 cc 1:500 + 0.15 cc antigen (1:7000)
0.05 cc 1:500 + 0.10 cc buffer + 0.15 cc antigen (1:3000)
(1:1000)
(1:3000)
0.15 cc 1:5000 + 0.15 cc antigen (1:10,000)
0.05 cc 1:5000 + 0.10 cc buffer + 0.15 cc antigen (1:30,000)
0.15 cc buffer + 0.15 cc antigen Control
0.15 cc buffer + 0.15 cc antigen Control
(1:10,000)
(1:30,000)

Mechanized and Animal Transportation in Modern Armies

Through the constant publicity given to the use of machinery by modern armies, the American people are being led to believe that horses and mules are no longer needed by the United States army. So deeply is this idea fastened to the public mind that the mention of animals in coming military operations is not taken seriously by the most thoughtful audience. Yet, with each increase in our military strength more horses and mules are procured; and, moreover, authentic information coming from both fronts of the present European conflict reveals that animals are being used on a large scale for artillery, cavalry, supplies and ambulance service, just as wise military men had predicted.

A recent bulletin of the Horse and Mule Association of America shows how wars have always increased the demand for horses and mules as well as for men. The present war is not apt to be an exception. Mules and horses are "implements of war" of the first rank and of the greatest importance. They differ from airplanes, trucks and tanks only in that they are living things.

Studies on the Immunology of Toxic Substances Produced by Streptococci* Isolated from Equine Encephalomyelitis†

By GEORGE T. KENSLER, Ph.D.

Vincennes, Ind.

ROSENOW and Schlotthauer[1] have reported the isolation of streptococci from the brains of horses in fatal cases of encephalomyelitis. The work reported in this paper was undertaken to determine if such streptococci produce toxic substances when grown *in vitro* and to investigate their immunizing properties.

DETERMINATION OF TOXICOGENIC STRAINS

In the first experiment Difco nutrient broth was utilized. Inoculations were made directly into flasks containing the broth from cultures maintained in a chick-mash medium. After ten days of incubation at 37° C. these were observed for contamination and passed through Chamberlain filters. These filtrates were tested for sterility and toxicity. The latter was determined by intraperitoneal injections of 1 mg. (0.015 gr.) of the filtrates into inbred white mice. These developed transitory reactions of extreme irritability, restlessness and a tendency to run in circles. Animals similarly inoculated with sterile broth manifested no symptoms.

Toxicity tests also were carried out by inoculating the chorioallantoic membrane of 12-day-old chick embryos with 0.3 mg. of the toxic filtrates. Examinations were made at intervals by using a candling lamp. Some of the filtrates caused the following sequence of changes. First, there was an apparent enlargement of the blood vessels in the membrane, *i.e.*, they became much more pronounced; next, the membrane became markedly transparent; and, finally, the embryo died. After death, the embryos were removed from the shells, keeping the chorioallantoic membrane intact. Macroscopic examination revealed that the blood vessels were contracted and a hemorrhagic ring was present in the membrane. Hemorrhagic areas also were noticeable in the region of the head and, sometimes, throughout the entire embryo. Uninoculated broth produced no reactions. By these methods five strains were found to produce toxic substances.

Another medium with which similar results were obtained consisted of veal infusion to which was added 1.5 per cent Difco proteose peptone, 0.5 per cent Difco bacto peptone, 0.5 per cent sodium chloride and 0.2 per cent dextrose. It was found, however, that by the addition of 0.25 per cent agar to this medium, substances lethal for mice as well as chick embryos could be obtained.

In the remainder of the work, only the strains of streptococci found to be toxicogenic were used in preparing filtrates. Preliminary experiments revealed that there was no significant difference between single-strain toxic filtrates and those from pooled cultures; therefore, only the latter were used.

The results of the filtrate-toxicity tests are recorded in table I.

TABLE I—Toxicity tests of equine encephalomyelitis streptococcal filtrates on white mice and 12-day-old chick embryos.

Filtrate No.*	1	2	3	4	5	6
Lethal dilution for mice†	0	0	1:2	1:2	0	1:2
Lethal dilution for embryos‡	1:0	1:2	1:4	1:4	1:2	1:4
Controls	Filtrates of uninoculated media, no reaction.					

*No. 1 = Difco nutrient broth, ten days of incubation; Nos. 2 and 5 = Veal infusion broth, ten days; Nos. 3, 4 and 6 = Semisolid agar, eight days.
†One mg. injected subcutaneously.
‡Three-tenths mg. inoculated on membrane.

It will be observed from the table that in the filtrates of the three broth media

*Cultures were kindly supplied by E. C. Rosenow of The Mayo Foundation, Rochester, Minn.

†This paper has been abstracted from a thesis submitted to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(1, 2 and 5) toxic substances lethal only for the chick embryos were present. The semisolid agar media (3, 4 and 6) contained toxic substances lethal for both.

TOXICOGENIC AND PATHOGENIC RELATIONSHIPS

Chick-mash cultures of the five strains of these streptococci also were inoculated into the chorioallantoic membrane of embryos. This was done to determine whether there is any relationship between their toxicogenicity and pathogenicity. There seems to be no correlation between the two. Two of these strains—2 and 5—were both toxicogenic and pathogenic. Strains 1, 3 and 4 were toxicogenic but not pathogenic.

Experiments were next carried out to ascertain the effect of immune serums on the filtrates.

ACTION OF IMMUNE SERUMS ON THE TOXIC FILTRATES

Chick embryos again were employed as indicators. Three types of serums were utilized: 1) A specific or streptococcic equine encephalomyelitis immune horse serum; 2) a nonspecific or streptococcic poliomyelitis immune horse serum* and normal horse serum. In all tests the serums and filtrates were used undiluted. The ratio between the two was varied, however, by adding to a constant volume of serum varying amounts of filtrate. Mixtures were kept at room temperature for 15 minutes before making inoculations. These tests were controlled by injecting chick embryos with filtrate and saline, and serum and saline.

The results were as follows: All toxic filtrates were unaffected by either the nonspecific immune or normal horse serum. They were rendered nontoxic by the specific immune serum, as evidenced by the survival of the embryos, when the ratio of filtrate to serum was no greater than 2 to 1. There was one exception, filtrate 5; with it the ratio was 3 to 1. Serum controls showed no reaction but all filtrate controls died.

*This serum was kindly supplied by W. A. Jamieson of the Eli Lilly Research Laboratories, Indianapolis, Ind.

Toxic filtrates also were obtained with streptococci isolated from cases of poliomyelitis. In similar tests, the toxicity of these filtrates was affected by streptococcic poliomyelitis immune serum but not streptococcic equine encephalomyelitis immune serum.

It was deemed advisable next to determine the antigenicity of these filtrates with mice. In order to make antigenicity evaluations it was first necessary to ascertain the minimum lethal dose of the virus (western strain, cultivated on chick embryo) for these animals.

DETERMINATION OF MINIMUM LETHAL DOSE OF CHICK-EMBRYO VIRUS

Serial dilutions of the virus were made in sterile physiological salt solution and duplicate mice were inoculated with 0.3 mg. intracranially with each dilution. The minimum lethal dose of virus was taken as the amount contained in the highest dilution causing death of the animals in three to four days.

IMMUNIZATION OF THE MICE

Filtrates.—An inbred strain of white mice weighing approximately 22 Gm. (0.7 oz). was used. Before use the filtrates were treated with 0.3 per cent formalin and incubated at 37° C. for four to five days to reduce their toxicity. Subcutaneous injections were made daily for five days. The initial injection was 0.2 mg. and each injection following was increased by 0.2 mg. Thus, a total volume of 3 mg. was given to each mouse. After a rest period of ten days they received ten minimum lethal doses of the western strain of equine encephalomyelitis virus intracranially.

It was not deemed advisable to determine the exact degree of immunity at this time. Ten minimum lethal doses were decided upon, since resistance to this amount of virus is a definite indication of immunity. This is the dose schedule usually employed for determinations of this kind.

Bacterial Vaccine.—For purposes of comparison, other antigens were utilized. A formalinized bacterial vaccine was prepared from cultures of the five strains.

Eighteen-hour broth cultures were centrifuged, the supernatant fluid removed and the bacteria resuspended in enough 0.3 per cent formalinized physiological salt solution to give a concentration of 8 billion organisms per mg. In order to kill the streptococci the vaccine was kept at incubator temperature for 24 hours. Injections were made every other day for a period of nine days. The dosage, beginning with 0.1 mg., was increased by 0.1 mg. at each succeeding inoculation. A total volume of 1.5 mg. was administered. The mice received ten minimum lethal doses of virus intracranially ten days after the last injection.

Bacterial Vaccine and Filtrate.—Similarly, another group of mice received bacterial vaccine immediately followed by an equal amount of formalinized filtrate.

*Chick-Embryo Vaccine.**—Another group was immunized with formalinized chick vaccine, western strain, in the following manner: An initial dose of 0.2 mg. injected subcutaneously was followed five days later with 0.3 mg.

After immunization each of the above groups of mice was tested for resistance to the virus.

RESULTS OF ANTIGENICITY TESTS

The results of intracranial injections of virus in mice following immunization with the various antigens are summarized in table II.

It will be observed from table II that the average protection induced by the filtrates was about 20.8 per cent. The bacterial vaccine gave the least protection. When employed with one of the filtrates, No. 2, immunity was not improved. The resistance produced by the chick-embryo vaccine was slightly greater than the average produced by the filtrates. It is quite evident that formalinized filtrates of the streptococcic cultures produce an immunity to the virus in mice.

TABLE II—Resistance of mice immunized with the filtrates, bacterial vaccine, filtrate and bacterial vaccine, and chick-embryo vaccine, to ten minimum lethal doses of the virus.

ANTIGEN USED	NO. OF MICE	NO. OF DAYS SURVIVED							PER CENT PROTECTED
		3	4	5	6	7	8	21*	
Formalinized filtrate No. 2	20	20	12	10	7	5	4	4	20.0
Formalinized filtrate No. 3	18	18	15	10	4			4	22.2
Formalinized filtrate No. 4	20	15	8	3				3	15.0
Formalinized filtrate No. 5	21	12	10	8	5			5	23.8
Formalinized filtrate No. 6	22	18	13	8	5			5	22.7
Bacterial vaccine	14	10	4	2				2	14.3
Bacterial vaccine and filtrate No. 2	20	12	8	4				4	20.0
Chick vaccine (western)	20	20	10	5				5	25.0
Virus controls	14	0						0	0.0

*Observed for 21 days.

SUMMARY

Some strains of streptococci isolated from equine encephalomyelitis produce filtrable toxic substances. These toxic substances can be titrated on chick embryos and white mice. An incubation period of eight to ten days was used for their production.

No relationship exists between toxicogenicity and pathogenicity, as determined on chick embryos. Filtrates are rendered atoxic by specific immune serum and formalin. Streptococci isolated from poliomyelitis also produce toxic substances. Formalinized filtrates and chick-embryo vaccine produce comparable degrees of immunity.

ACKNOWLEDGMENT

Sincere acknowledgment is hereby made of the helpful supervision and constructive

*Supplied through the courtesy of Joseph Zichis of the Illinois State Department of Health, Chicago, Ill.

EDITORIAL

What is not interesting is that which does not add to our knowledge of any kind; that which is vaguely conceived and loosely drawn; a representation which is general, indeterminate, and faint, instead of being particular, precise, and firm.—Matthew Arnold, 1853.

Artificial Insemination

FROM MANY sources come warnings that unless we increase our interest in artificial insemination, this highly touted procedure will fall into outside hands.

There is wisdom in the warnings because this substitute for natural copulation involves delicate biological processes that even the most learned students of reproductive physiology do not understand. However, professional men are not as apt as laymen to be swept off their feet when new medical inventions come along. As a consequence, the interest of practicing veterinarians in the recent revival of this century-old substitute for coitus has not been excited to the same extent as that of the breeders of live stock, who seem to have taken artificial insemination bait, hook and sinker as the salvation of their enterprise, far ahead of their knowledge of the subject.

Aside from the intricate technical phase of the subject, it is indeed doubtful whether there is any economy in the practice in a country where sires are not scarce; and, as Williams points out, in prolonging the usefulness of physically incapacitated sires, it is evident that such animals have lost their virtue as producers of satisfactory progeny. The breeding history of herds created by artificial insemination must first be of record before the wise veterinarian will recommend its substitution for normal copulation. When that time has arrived, the expert who knows all of the pitfalls lying along the reproductive process will not have lost prestige by not having lost his head at the start and, certainly, the scientific side of the procedure will never be simplified into an everyday travail of the layman.

There are places for artificial insemination in animal production. But, just now it is doubtful indeed whether shipping semen from one farm to another, perhaps many miles away, is one of them. The effect of longtime preservation on the vigor of semen remains to be determined, and it will not be determined until reliable records of several generations are available.

When several animals come in heat at the same time *sur place*, one ejaculation may be transplanted from one to the others to excellent advantage. In that event, one copulation is preferable to two or more. The drain upon the reproductive vigor of the sire is reduced, mathematically, and the presumption is that the handling of the semen for that purpose inflicts minimum damage to its wiggling components.

The idea we wish to convey is that artificial insemination has a place in the practice of veterinary medicine only to the extent of our knowledge of its many details, all along the road from semen to the reproductive ability of adult life. If veterinarians aid breeders in executing the art, that is justified veterinary practice, but in doing so, the wise veterinarian will not overlook the basic principles of mammalian reproduction.

The problem of storing and shipping semen is still in the experimental state. Although the life of spermatozoa has been preserved for periods of over 500 hours and

(*Continued from preceding page*)

criticisms of C. A. Behrens, professor of bacteriology, Purdue University, Lafayette, Ind.

Reference

[1]Rosenow, E. C., and Schlotthauer, C. F.: Proc. Staff Meet. Mayo Clinic (Oct. 6, 1937).

fertilization has been obtained from semen several days old, the sequences have not been encouraging. They vary from nonviable young and abortions to total failure. Moreover, breeding histories of the progeny are not of record.

In a detailed report on "Storage and Shipment of Semen," Professor Bonadonna of the Experimental Institute for the Artificial Insemination of Domestic Animals points out (*The Veterinary Record,* August 19, 1939) that the survival of sperms varies with species, breeds, strains and individuals and such other factors as the health, sexual vigor and age of the sire, together with quantity and kind of accessory secretions and meticulous technics. The limits of storage that have proved to be useful are:

Cattle	50 to 120 hours
Sheep	70 to 120 hours
Horses	12 to 24 hours
Hogs	1 to 2 hours

For practical purposes, however, Bonadonna would restrict the duration of storage to:

Cattle	12 to 30 hours
Sheep	30 to 50 hours
Horses	12 to 24 hours
Hogs	Immediate use

W. L. Williams, in his revision of *Diseases of the Genital Organs of Domestic Animals,* declares that the subversive effect of the handling of semen may extend far beyond the birthline; in other words, through life.

Seals of Approval

THE FIRST case against a publisher for violating the Wheeler-Lea amendment to the Federal Trade Commission Act is being fought out in the courts. The outcome is awaited with considerable anxiety, for if the FTC is upheld, it will likely go on prosecuting other magazines for publishing false and misleading advertising. The defendant is *Good Housekeeping,* published by Hearst Magazines, Inc. The charge is printing in its advertising pages misleading and grossly exaggerated claims for certain products. The suit "burst like a bombshell among ad-men and popular magazine editors during the later days of August," says *Consumers' Digest* for November.

Specifically, *Good Housekeeping* is charged with claiming that certain articles carrying seals of approval have been scientifically tested, when, as a matter of fact, they allegedly have not been tested and approved by any scientific laboratory. The products in limbo are mainly cosmetics.

"Doctors"

JUDGING from dialogues seen in current medical magazines, some physicians are quite perturbed over the use of "doctor" by dentists *et al.* Veterinarians, of course, are in the "et al," and there is no escape, as much as we may try.

We are hopeful that the readers of the JOURNAL will approve the dropping of "Dr." from almost every name we print. The change from habits of the past is intended to save a lot of printer's ink if not also to seem less ridiculous. The notion of our sensitive friends in the field of human medicine that the handle is exclusive property has nothing to do with the change. Nor is the veterinarian claiming that premedic plus and four years entitles him to stick the handle in front of his name. The handle is yours to handle, Doc, but remember, there are oodles of pioneering to do in the U. S. A. before the gamin (and others) will stop hollering "Hello Doc" to the physician and the dentist and the druggist and, of course, the fellow who watches over the community live stock.

Anyhow, we do not want to confuse the conservation of the nation's food supply with such ordinary things as appendicitis, toothache and mixing drugs.

Apothecary and Metric Units in Future Issues

AS FAST as material already set in type is used up, the JOURNAL will designate weights and measures in the metric system with parenthesized equivalents after each figure. Thus: 1 Gm. (15 gr.), 1 cc. (15 mm.), 30 cc. (1 oz.), 1 kg. (2.2 lb.), etc. Capital "G" will be used in abbreviating the gram in order to avoid confusing its abbreviation with that for the grain.

The apothecary figures used in the paren-

theses will be approximate except where precision is important, such as is generally the case in laboratory work or where accurate dosage is essential. In these events decimals will be used to express the exact figure.

The object of this change is to overcome the growing custom of using both systems in the same article, the same paragraph or, as is often the case, in the same sentence. The faults of American veterinary authors in this respect have been amazing and, in scanning through manuscript after manuscript from every source, we find that the habit is not decreasing.

Veterinary Colleges and Wild Animal-Disease Education

IN THE EVOLUTION of veterinary medicine well-defined stages of development have taken place. New activities have been incorporated in the work of those engaged in research on animal diseases as well as the larger group who practice the art of healing. Correspondingly, all other scientific groups have adjusted their endeavors to meet changing economic and social conditions. Many of these groups now function in a manner much unlike that of their founders.

In our own generation we have seen greatly increased values placed upon pet stock and poultry, with the result that veterinarians are rapidly turning their attention to these specialties. At the present time fur-farming and game-bird-production interests frequently seek veterinary counsel, and outbreaks of disease in wild game and waterfowl have been brought to the attention of our profession. Too often, however, the approach made by those consulted shows a lack of the basic training required in the handling of such problems.

State agricultural colleges are attempting to cover this field in courses given to students of biology who are training for game-management careers. These courses are designed to teach the students to recognize the conditions that commonly cause wildlife losses. Attention is given to etiology, symptoms, lesions, and corrective measures. In the broad program of wildlife conservation that is being conducted by various state and federal agencies, one can not find fault with this kind of training for the custodians of our wildlife assets.

The question arises, "What are the veterinary colleges doing to supply similar, or more complete, information to veterinary students?" In a survey made recently by a veterinarian whose graduate school work was done in the field of wildlife diseases, it was pointed out that few veterinary colleges in this country devote any organized effort to fortify the students with adequate information on wildlife pathology. On fur and game farms there is almost constant need for veterinary services, and veterinarians frequently can aid conservationists to determine the cause of loss among animals in the wild.

Who Will Be the Food Inspector of the Future?

UNLESS we go on pursuing the policy of curtailing the number of veterinarians to such a degree that a new profession will have to be developed to safeguard the people's food supply, the veterinarian, unchallenged, will be the food inspector of the future. The reason for this hopeful prophecy lies in the training that a well-qualified food inspector must have in order to understand this important art.

Right now, keen observers are watching an entirely new profession climb to social significance, with "sanitary engineers" and "practical meat-and-milk inspectors" furnishing the momentum of its progress. Few veterinarians can be found on the roster of this new undertaking; those who are enrolled occupy subordinate positions.

There would be no need of "sanitary engineers" and "practical meat-and-milk inspectors" (who are more or less politically obligated) if the civilian food-inspection service were planned after that of the army, which employs veterinarians to safeguard the food of the soldiers. The present population, faced with a deluge of dangerous alimentation, is becoming food-conscious and, like the army, eventually will demand

Reflections of the Memphis Session

Chapter II — Reviews of Committee Reports

Education

An analysis of the educational situation read by Chairman Mayo of the Committee on Education gives the number of students in the twelve veterinary schools of North America as 2,180 for the year 1938-1939. The figure does not include 590 pre-veterinary students. Of these, 395 were graduated by the ten schools of the United States and 74 by the two Canadian schools.

The Committee signalized the limitation of enrollment due to lack of physical equipment and teachers, and the tendency of state legislatures to keep the appropriations for veterinary education down to the needs of the states in which the colleges are located. The consequence is that the young men of 38 states are barred to a certain extent from entering the veterinary profession. The practice of permitting members of faculties to engage in private practice is frowned upon as "likely to cause jealousy of those not so forward" and criticism by private practitioners infringed upon.

The data collected on the average "student clock-hour load" per full-time teacher are set down in a group of revealing tables, and the qualifications of teachers as shown by their graduate degrees are analyzed.

Public Relations

The Committee on Public Relations laid down plans for expanding the excellent work carried out in recent years. Past achievements publicized on a nationwide scale through complimentary editorials and news releases have given the work of this committee a place of preëminence. Its activities will henceforth be cleared through the central office in order that all promotional work may be systematically coördinated.

The report recommends that the Association publish a popular magazine for public circulation—a "Hygeia" for the veterinary profession. Definite plans for starting this project are laid down in the report.

The relations of the veterinary profession to the agricultural extension service are extensively particularized in a historical survey covering 150 years. This phase of the report tends to clarify a perturbing factor in the development of veterinary science. Elaborate tables and figures attesting to the diligence and detailed efforts of this committee are a cool reminder to the veterinarians of this country that the future of the profession may well be entrusted to the national association.

The Committee proposes measures for the improvement of veterinary practice laws and for guiding the evolution of so-called state veterinary medicine, since much of the veterinary service in the future, as in the past, comes under that classification.

The profession is again reminded that it must somehow become a "homogeneous group" within which all branches work harmoniously to the same end. Justly, the official journal is criticized for failing to have discussed openly the problems of the profession for the general edification of its members. In short, the majestic work of the Association has been hidden away in small type pages and never again brought to the members' attention. Thus, the march of veterinary medicine has lagged while the more resourceful nonmedical groups profoundly interested in animal diseases have won a place in the hearts of the people, a place that rightly belongs to the veterinary profession. Readers interested in their own personal welfare should turn to pages 579-597 of the November issue, as therein is one of the many reasons why the development of a large and influential national

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(Continued from preceding page)

experts to protect its food supply. Whether these experts are going to be veterinarians or members of an entirely new profession depends considerably upon the resourcefulness of the veterinary profession.

organization is the paramount issue of the hour.

FOOD HYGIENE

Chairman Hardenbergh of the Committee on Food Hygiene read a comprehensive report on the subject into the Association's archives. Although 60.9 per cent of urban centers of over 1,000 population have some sort of milk inspection, 55.1 per cent of the inspectors as a whole have no college degree. The higher the population, the smaller is the proportion of professional men employed in this work. Physicians and veterinarians in small municipalities seem to be more interested in milk inspection than those of the larger cities. The figures are 55.6 per cent of professional men for small cities and but 21.6 per cent for the larger ones.

The Committee describes the qualifications of milk inspectors in detail. In general, the requirement is a degree in medicine, veterinary medicine, sanitary engineering, agriculture or other allied branch of learning. Clearly, the report shows the need of an adequate personnel sufficiently trained to carry out the purposes of this branch of the public health service. [*Recently, when the Illinois department of health undertook to enforce the new "Grade A milk laws," it was found that the available qualified personnel was inadequate.*] In this respect, the Committee on Food Hygiene contradicts the Committee on Education. The former recommends the expansion of milk-inspection services, which means the need of more professional men, whereas the latter believes that the consense is in favor of limiting the enrollment of veterinary students. As these two committees represent basic projects, the need of an intercommittee conference seems important. The discord is but one of many which have led the Committee on Public Relations to point out the bewildering dissensions and lack of accord among the component groups of the profession.

BANG'S DISEASE

Chairman Donham registers an understandable review of a gigantic project—the eradication of brucellosis in animals, a subject growing more and more significant in animal production and public health.

In round numbers, 21 per cent of the cattle of the United States are under supervision and 650,000 blood tests per month were made during the year (1938). In seven states more than 50 per cent are under supervision. . . The federal government has taken control of the antigens used by supplying them for both tube and plate tests. . . The field veterinarian should be held responsible for the diagnosis. . . The improper use of live-culture vaccinations is detrimental to the progress of the prevailing plan of Bang's disease control. A plan of combining the test-and-slaughter method with calfhood vaccination is in the offing and should be worked out, since eradication is the objective of both plans. . . Additional researches on Bang's disease are advisable, particularly in connection with the infection in farm animals other than cattle. Sheep may be an important reservoir of the organism. . . In the demesne of public health there is no longer any doubt that undulant fever is contracted only from animals and, therefore, imposes a great responsibility upon the veterinary service. . . The confusing lack of uniformity of state laws has aroused justifiable indignation among cattle owners. Revision of outmoded livestock sanitary laws is an outstanding obligation.

Since all veterinarians are intimately concerned in brucellosis of animals and man, a careful reading of this report on pages 606-611 of the November issue is certainly in order.

POULTRY DISEASES

With the interest in poultry medicine mounting rapidly it is obvious that the report of this committee adds valuable pages to the Association's records. Outstanding in this report are 1) the compilation of 101 references on virus diseases and 2) continuation of the Committee's undertaking to establish a uniform nomenclature of poultry diseases—a task which, hereafter, will be placed in the hands of a special committee for completion. A glance through these two parts of the report

attests to the painstaking work that this committee is doing.

Among the subjects receiving special mention are 1) paratyphoid infections of chickens and turkeys, 2) pullorum disease, 3) equine encephalomyelitis in birds, 4) avian lymphomatosis, and 5) differentiation of respiratory diseases. The Committee points out that while pullorum disease control is making progress under "the poultry improvement plan," the basic measures vital in the eradication of infectious diseases are apt to be overlooked to the detriment of the project. Fowl paralysis continues to be a major problem of poultry production. Its etiology remains mysterious. Neither bacterial, virus nor nutritional agencies have yet been incriminated and the present work in the research field is directed toward improving the vitality of chickens through studies of the pathologic and genetic aspects of the disease. The importance of our differentiating respiratory diseases before hurriedly resorting to the vaccination against laryngotracheitis is emphasized.

[*Reviews of committee reports will be continued in future issues.*]

The Revised Code of Ethics

SINCE the revised code of ethics will be declared in the January issue for action at the Washington, D. C. (1940) session, it should be studied and frankly discussed at the coming meetings of the component associations. The delegates to the House of Representatives should come to the Washington meeting prepared to vote understandingly on important planks of the new document.

In the matter of advertising in telephone directories, for example, the Association has arrived at a cross road where it must declare itself for or against display advertisements, or pursue the more timid course of *laissez faire* and allow the membership to do as it pleases in that respect. Opinions should now be mobilized and action taken one way or the other before that reduviid becomes inseparably attached to the customs of the service.

Except here and there in a few of the larger cities, advertising by practitioners in telephone directories and other media is not decreasing. Rather, the practice is increasing. In the smaller towns the opening of almost every small animal hospital seems to be the signal to start advertising campaigns. The general practitioner who never thought of advertising quickly gets the habit, since the canine specialist in the smaller centers of population competes for large animal practice. Thus, advertising is spread farther and farther into the one-time ethical precincts.

Contrary to expressed beliefs, medical ethics are not relaxing. They are becoming more rigid, except in the veterinary profession. The House of Representatives is therefore obligated to make an important decision this year. It should be a decision of the firm but fatherly kind with the welfare of the veterinary service uppermost in mind.

Fuadin—a Correction

TO A contributed article on the treatment of heartworms in dogs, published in the November 1939 issue, pp. 566-569, there was appended a note that requires clarification.

It was stated that Fuadin is a foreign-made preparation, whereas, in fact, it is strictly an American-made product. Naturally, of course, the value of a medicinal preparation can not be measured by its country of origin, for medicine has never known any national boundaries. Fuadin has been used in human and veterinary medicine for many years, and has been accepted and described in detail by the Council on Pharmacy and Chemistry of the American Medical Association.*

To clarify any misunderstanding that might be prompted by the note, attention is called to the fact that the statement of efficiency reports contained therein does not represent the views of the JOURNAL and that there is no published evidence based on a study of Fuadin as compared with Stibsol, the product discussed in the November issue, which indicates the effectiveness of one as against the other.

*Jour. A.M.A., cxiii (Sept. 9, 1939), p. 1033.

APPLICATIONS

Thirty-three per cent of the applicants for membership this month were graduated 15 to more than 30 years ago. That these men see fit to enroll after such a long interval is a significant indication of the Association's expanding influence.

First Listing*

ALKIRE, ROBERT LOUIS
Grantville, Md.
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BADGLEY, FRANCIS ROY
39 Cedar Ave., Farmingdale, N. Y.
D. V. M., Cornell University, 1939. Vouchers; K. F. Hilbert and Herman Tax.

BAHENSKY, MELVIN DONALD
Palmer, Neb.
B. V. Sc., Ontario Veterinary College, 1939. Vouchers: J. E. Weinman and S. E. Hershey.

BARTA, JAMES CHESTER
2916 S. E. 36th Ave., Portland, Ore.
D. V. M., Kansas City Veterinary College, 1917. Vouchers: Sherman L. Brown and E. E. Chase.

BEADNER, HAROLD
Box 44, Coquille, Ore.
B. S., D. V. M., Washington State College, 1939. Vouchers: Charles H. Seagraves and L. A. Merillat.

BROOKS, WILLARD C.
Prairie Grove, Ark.
D. V. M., Texas A. & M. College, 1939. Vouchers: C. D. Stubbs and C. L. Baker.

BROUSSARD, GEORGE P.
417 E. Main St., New Iberia, La.
D. V. M., Texas A. & M. College, 1939. Vouchers: W. A. McDonald and L. A. Merillat.

BROWN, JAMES ROBERT
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V. M. D., University of Pennsylvania, 1937. Vouchers: Edmond C. Porter and James E. Burton.

BULGER, EDWARD CAMPBELL
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V. M. D., University of Pennsylvania, 1938. Vouchers: C. Thibeault and Myron S. Arlein.

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D. V. M., McKillip Veterinary College, 1917. Vouchers: E. P. Snortum and W. L. Curtis.

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D. V. M., Kansas City Veterinary College, 1914. Vouchers: H. A. Seidell and L. A. Merillat.

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D. V. M., Michigan State College, 1935. Vouchers: E. C. W. Schubel and J. C. Schwabland.

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B. S., University of Idaho, 1932, D. V. M., Iowa State College, 1936; M. S., University of Idaho, 1933. Vouchers: A. K. Kuttler and E. T. Baker.

*See January 1939 JOURNAL.

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Thimmig, John W., Brighton, Colo.
Tower, William James, 637 Main St., Leominster, Mass.
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Zlotnick, Albert Morris, 3726 Riverdale Ave., Bronx, N. Y.

The amount that should accompany an application filed this month is $5.42. This covers membership fee and dues to January 1, 1940, including subscription to the JOURNAL. It is suggested that applications filed this month be accompanied by a remittance of $10.42, the additional $5.00 being for the 1940 dues.

Veterinary Literature

NOT ONLY in this country but everywhere, veterinary literature is a mess. If the *Lancet* (London) was inspired to focus the medical eye on "The Mess of Medical Literature," what should be the legend for a similar editorial on the literature of the veterinary profession? A correspondent of the *Journal of the American Medical Association* says that there are between 30,000 and 40,000 scientific magazines in the world, three fourths of which should not be published. Obviously, this critic would place most of the veterinary journals in that group, as they have both the qualitative and quantitative faults of which he speaks.

The sarcastic philosopher who wrote, "Don't write, someone may believe you," may not have been thinking of scientific periodicals, but the warning does apply to the mass of periodical literature circulated *ad captandum vulgus* among veterinarians. This is not a slap at any periodical or group of periodicals but a hint to all, including ourselves, that those who publish scientific literature voluntarily assume a sacred responsibility they have no ethical right to misuse.

Speaking of our profession alone, some sort of control agency should govern literary production and then undertake the important task of indexing the output and of preventing unnecessary overlapping. Such a service and advisory council should somehow be developed through the instrumentality of the A.V.M.A.

It is estimated that water pollution in the United States causes a loss of six billion dollars per annum.

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Harrie W. Peirce, 22 Gleason St., West Medford, Mass. (1944)

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E. T. Hallman, Michigan State College, East Lansing, Mich.
E. T. Faulder, 399 State St., Albany, N. Y.
Jacob Traum, University of California, Berkeley, Calif.

Bang's Disease

R. R. Birch, *Chairman*, Cornell University, Ithaca, N. Y.
Adolph Eichhorn, Animal Disease Station, Beltsville, Md.
Chas. P. Bishop, State Department of Agriculture, Harrisburg, Pa.
C. R. Donham, Ohio State University, Columbus, Ohio.
Hugh S. Cameron, University Farm, Davis, Calif.
R. A. Hendershott, State Department of Agriculture, Trenton, N. J.
W. Wisnicky, University of Wisconsin, Madison, Wis.

Interstate Shipment of Live Stock by Truck

J. L. Axby, *Chairman*, 209 State House, Indianapolis, Ind.
V. S. Larson, 928 Lake Court, Madison, Wis.
H. C. Rinehart, Route 5, Box 187, Springfield, Ill.
H. A. Seidell, 237 Franklin Ave., Des Moines, Iowa.
H. E. Curry, Jefferson City, Mo.

Twelfth International Veterinary Congress Prize

Cassius Way, *Chairman*, 25 Vanderbilt Ave., New York, N. Y.
H. W. Jakeman, 44 Bromfield St., Boston, Mass.
John R. Mohler, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.
Col. R. A. Kelser, Office of the Surgeon General, U. S. Army, Washington, D. C.
I. E. Newsom, Colorado State College, Fort Collins, Colo.

Local Arrangements for Washington, D. C., Meeting, 1940

John R. Mohler, *Chairman*, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.
Adolph Eichhorn, *Associate Chairman*, Animal Disease Station, Beltsville, Md.
I. M. Cashell, *Secretary*, 2807 - 18th St., N.W., Washington, D. C.
George W. Gillie, *Publicity*, House Office Bldg., Washington, D. C.
H. M. O'Rear, *Entertainment*, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.
Mrs. Clifton D. Lowe, *Ladies' Entertainment*, 3429 Oakwood Terrace, N.W., Washington, D. C.
Harry W. Schoening, *Educational Exhibits*, Bureau of Animal Industry, Washington, D. C.
Col. J. F. Crosby, *Technical Exhibits*, Office of the Surgeon General, Washington, D. C.
Col. R. A. Kelser, *Banquet*, Office of the Surgeon General, Washington, D. C.
A. E. Wight, *Alumni Dinners*, Bureau of Animal Industry, Washington, D. C.
John P. Turner, *Large Animal Clinic*, 1357 Kennedy St., N.W., Washington, D. C.
Mason Weadon, *Small Animal Clinic*, 2119 - 18th St., N.W., Washington, D. C.
Willard H. Wright, *Reception*, National Institute of Health, U. S. Public Health Service, Washington, D. C.
Walter J. Hall, *Poultry and Sheep Clinic*, 5 Pine St., College Heights, Hyattsville, Md.

Sub-Committee on Veterinary Items National Formulary Committee (Ten-Year Appointment)

H. D. Bergman, *Chairman*, Iowa State College, Ames, Iowa.

R. F. Bourne, Colorado State College, Fort Collins, Colo.
P. W. Burns, A. & M. College of Texas, College Station, Texas.
C. F. Cairy, Michigan State College, East Lansing, Mich.

Representatives

National Research Council

W. W. Dimock, University of Kentucky, Lexington, Ky.

Advisory Board, Horse and Mule Association of America

T. A. Sigler, Box 250, Greencastle, Ind.

American Association for the Advancement of Science

Ward Giltner, Michigan State College, East Lansing, Mich.

Resident State Secretaries

Alabama: R. L. Mundhenk, Alabama Polytechnic Institute, Auburn.
Arizona: J. B. McQuown, Box 1905, Tucson.
Arkansas: R. W. Williams, Box 923, Eldorado.
California: W. L. Curtis, 1264 W. 2nd St., Los Angeles.
Colorado: J. H. Bouton, 9357 E. Colfax Ave., Aurora.
Connecticut: Geo. E. Corwin, 269 State Office Bldg., Hartford.
Delaware: C. C. Palmer, University of Delaware, Newark.
District of Columbia: W. M. Mohler, 5508 Nebraska Ave., N.W., Washington.
Florida: M. W. Emmel, University of Florida, Gainesville.
Georgia: Chas. C. Rife, 420 Edgewood Ave., S.E., Atlanta.
Idaho: A. K. Kuttler, 312 Federal Bldg., Boise.
Illinois: W. B. Holmes, 225 E. Washington St., Springfield.
Indiana: J. C. Schoenlaub, 1026 N. Illinois St., Indianapolis.
Iowa: A. H. Quin, 313-49th St. Place, Des Moines.
Kansas: R. F. Coffey, Johnson Serum Co., Crane and Jefferson Sts., Topeka.
Kentucky: F. E. Hull, University of Kentucky, Lexington.
Louisiana: W. T. Oglesby, Louisiana State University, University.
Maine: Myron E. Maddocks, 2 Brooklawn Ave., Augusta.
Maryland: Mark Welsh, College Park.
Massachusetts: L. A. Paquin, Box 225, Webster.
Michigan: C. F. Clark, 1100 Burcham Drive, East Lansing.
Minnesota: John N. Campbell, Truman.
Mississippi: W. L. Gates, Box 417, Clarksdale.
Missouri: Harvey W. Young, 3634 Main St., Kansas City.
Montana: W. J. Butler, Capitol Station, Helena.
Nebraska: J. E. Weinman, 2525 O Street, Lincoln.
Nevada: Edward Records, University of Nevada, Reno.
New Hampshire: F. F. Russell, 286 Pleasant St., Concord.
New Jersey: J. R. Porteus, Box 938, Trenton.
New Mexico: T. I. Means, Box 1174, Santa Fe.
New York: C. P. Zepp, 136 W. 53rd St., New York.
North Carolina: J. H. Brown, Tarboro.
North Dakota: Frederik Low, Box 348, Oakes.
Ohio: W. F. Guard, Ohio State University, Columbus.
Oklahoma: S. E. Douglas, 124 N.W. 23rd St., Oklahoma City.
Oregon: Charles H. Seagraves, 1514 Washington St., Oregon City.
Pennsylvania: A. H. Craige, Jr., University of Pennsylvania, Philadelphia.
Rhode Island: J. S. Barber, 310 State House, Providence.
South Carolina: W. A. Barnette, Greenwood.
South Dakota: Geo. E. Melody, Gettysburg.
Tennessee: John H. Gillmann, 769 Vance Ave., Memphis.
Texas: J. Gilbert Horning, 3611 Willia St., Houston.
Utah: E. D. Leiby, 2363 Jefferson Ave., Ogden.
Vermont: G. N. Welch, 43 Union St., Northfield.
Virginia: A. J. Sipos, 1102 State Office Bldg., Richmond.
Washington: R. A. Button, 2909 S. M St., Tacoma.
West Virginia: S. E. Hershey, Box 293, Charleston.
Wisconsin: J. S. Healy, 330 Federal Bldg., Madison.
Wyoming: H. D. Port, 304 Capitol Bldg., Cheyenne.

Resident Territorial Secretaries

Alaska: J. B. Loftus, Juneau.
Canal Zone: T. L. Casserly, Box 3035, Cristobal.
Hawaii: L. E. Case, 3851 Woodlawn Drive, Honolulu.
Philippine Islands: A. K. Gomez, College of Veterinary Science, Pandacan, Manila.
Puerto Rico: Francisco Menendez, Box 819, San Juan.
Virgin Islands: Gordon Kendell, Agricultural Experiment Station, Christiansted, St. Croix.

Resident Provincial Secretaries

Alberta: J. C. Hargrave, Box 673, Medicine Hat.
British Columbia: W. R. Gunn, Live Stock Branch, Department of Agriculture, Victoria.
Manitoba: R. H. Lay, 613 Dominion Public Bldg., Winnipeg.
New Brunswick: T. Fred Johnston, 117 Leinster St., St. John.
Nova Scotia: George Townsend, Box 76, New Glasgow.
Ontario: W. Moynihan, 366 Keele St., Toronto.
Quebec: A. A. Etienne, Montreal.
Saskatchewan: Mark Barker, P. O. Bldg., Regina.

Foreign Corresponding Secretaries

Argentina: Willy Rucks, Valle 1314, Buenos Aires.
Bahamas: James N. Brown, Box 242, Nassau, N. P. Island.
Bermuda: C. J. Cooper, Hamilton.
Chile: Julio San Miguel, Casilla 537, Santiago.
China: Ching Sheng Lo, Soochow.
Cuba: B. J. Crespo, Apartado 246, Havana.
Denmark: Hans C. Bendixen, Magnoliavej 53, Copenhagen.
Dominican Republic: G. A. Roberts, Apartado 1311, Ciudad Trujillo.
England: T. P. White, c/o American Consulate, 1 Grosvenor Square, London.
Germany: Prof. Oskar Seifried, Institut für Tierpathologie d Univ. Veterinarsrasse 6, Munich.
Hungary: Alexander Kotlan, Royal Hungarian Veterinary College, Budapest VII.
Jamaica: Stephen Lockett, Department of Agriculture, Hope, Kingston.
Mexico: Luis Santa Maria, Apartado Postal, 2067, Mexico, D. F.
New Zealand: Wm. C. Ring, 16 Wyndham St., Auckland.
Peru: James F. Mitchell, c/o Hacienda Pachacayo, Pachacayo.
Scotland: A. W. Whitehouse, Glasgow Veterinary College, 83 Buccleuch St., Glasgow.
Union of South Africa: G. Martinaglia, Box 1620, Johannesburg, Transvaal.
Straits Settlements: Leonard L. Newman, Singapore Dairy Farm, Bukit Panjang, Singapore.

Administrative By-Laws

Article I

Corporate Officers

Section 1.—The officers having immediate charge of the Association's affairs in its corporate capacity shall consist of a president, a president-elect, a first vice-president, an executive secretary, a treasurer, and an executive board of 13 members. They shall be known as the corporate officers of the Association.

Section 2.—The specific method of election, the tenure, the duties and obligations, and replacement of corporate officers shall be described in these administrative by-laws.

Section 3.—No member shall be elected as a corporate officer until he has been a member in good standing for at least two years preceding the date of his election; the president-elect for at least five years.

Section 4.—Subject to the restrictions hereinafter prescribed, the corporate officers shall have full charge of the management of the Association.

Article II

The President

Section 1.—The president shall be the chief executive officer of the Association.

Section 2.—Election (See article III, section 1).

Section 3.—Duties:

a) The president shall preside at all general sessions and at all sessions of the House of Representatives but is empowered with the authority to yield the chair to the senior vice-president available for the performance of these duties;

b) he shall deliver an address at the regular annual session on matters pertaining to the advancement of veterinary science;

c) he shall appoint all regular and special committees and shall promptly fill vacancies in the membership of committees created by any cause;

d) he shall serve as a member of the Executive Board *ex-officio* with the same franchise as its other members; he shall be a member of the Board of Governors; and

e) he shall be considered as an *ex-officio* member of all committees with full power to supervise and direct their work. The president shall, however, not be empowered with authority to suppress committee reports, that authority being delegated to the Executive Board and House of Representatives.

Section 4.—Tenure: The president's term of office is one presidential year, which shall be defined as the interval between the ends of two consecutive annual sessions. In event of an emergency not provided for in this constitution and administrative by-laws, he shall hold office until a successor has been legally installed. (See article III, section 4.)

Article III

The President-Elect

Section 1.—Election: The president shall be elected by ballot at a regular annual session in accordance with customary parliamentary procedure, or by *viva voce* vote when but one member is nominated for the office.

The nominations for the office of president-elect shall be made on the first day of a regular annual session. When more than one member are nominated, the election shall be held on the day next to the day of nomination, under the supervision of a board of three tellers, appointed by the president, who shall vouch for the eligibility of the voters in regard to membership and standing.

The polls shall be open in the executive secretary's office of the session from 9.00 a. m. to 6:00 p. m. on the day next following the date of nomination. The executive secretary shall furnish a sealed ballot box into which all ballots will be dropped by the voters. The board of tellers shall count the ballots in the presence

of such nominees and members of the Executive Board as may desire to be present; and they (the tellers) shall announce the result of the vote at the general session next following the date of the election. In the case of a protest by one or more nominees made before the general session at which the tellers' report is made, the president is authorized to order a recount of the ballots, to be made by a committee of three —one from the board of tellers, one from the Executive Board and one from the general membership in attendance at the meeting.

These rules governing the election of the president-elect shall be announced to the floor of the general session preceding the election by the executive secretary or his legal representative.

Section 2.—Duties: The president-elect shall be a member *ex-officio* of the Executive Board with the same franchise as its other members. He shall be a member of the Board of Governors. (See article VI, section 5, of the constitution.)

He shall coöperate in the promotional work of the Association under the direction of the corporate officers, particularly in its relations to component associations, other scientific societies and student chapters.

The Committee on Budget shall appropriate a stipulated sum to defray the expense of the president-elect which is incurred in the line of duty.

Section 3.—Tenure: The president-elect shall hold office as such until installed as president. He shall be installed as president at the termination of the annual session next following the one at which he was elected.

The reëlection of the president-elect is prohibited.

Section 4.—Should the inability of the president-elect prevent his installation into the office of president in the manner prescribed in section 3 of this article, his successor shall be elected in the manner prescribed in section 1 of this article. In this event, the newly elected president-elect shall be installed as president at the end of the annual session at which he is thus elected.

Article IV

Vice-Presidents

Section 1.—Election: The five vice-presidents provided for in section 1, article VII of the constitution shall be elected at each regular annual session immediately following the election of the president-elect and as specified in section 1, article III. The vice-presidents thus elected shall be designated first vice-president, second vice-president, third vice-president, fourth vice-president, and fifth vice-president and shall be rated on a descending scale in the order named according to the number of votes received. When but one nomination is made, vice-presidents may be elected on a *viva voce* vote taken at the time of their nomination. The seniority rating of vice-presidents elected *viva voce* shall conform with the order in which they were nominated.

Section 2.—Duties: Vice-presidents shall perform the usual duties of that office customary in parliamentary procedure, that is, they shall function for the president in his absence from the chair, serving in the order of their seniority.

The first vice-president shall become president to fill out the unexpired term in the case of death or total disability or resignation of the president. He shall be duly installed into the office of president in any of the aforesaid events by the chairman of the Executive Board.

Section 3.—The first vice-president shall be a member of the Committee on Budget.

Section 4.—In principle, it is the sense of the Association, although not constitutionally mandatory, to choose the vice-presidents from prominent veterinarians located in widely separated geographic districts.

Article V

Executive Secretary

Section 1.—The executive secretary shall be an employé of the Association charged with carrying out the direction of the Board of Governors provided for in section 5, article VI of the constitution.

Section 2.—Election: The executive secretary is elected by the Board of Governors (composed of the chairman of the Executive Board, the president and the president-elect) acting for, and under the direction of, the Executive Board.

The report of his election shall be included in the annual report of the Executive Board to the House of Representatives and shall be subject to the approval of both of these bodies.

Section 3.—The executive secretary shall be the general manager of the Association. He shall direct the entire business of the Association, including the clerical work of the Association and of the Executive Board.

Section 4.—He may act as editor of the JOURNAL and may, upon approval of the Executive Board, employ such editorial and secretarial assistance as the Executive Board may deem necessary for the conduct of his office.

The executive secretary shall present a written report of his activities and of the Association's affairs at each regular annual session of the Association.

He shall furnish the Executive Board a bond of such amount as said Board may prescribe.

Article VI

Treasurer

Section 1.—Election: The treasurer shall be elected by ballot at each regular annual session at the time and in the manner designated in section 1, article III for the election of the president-elect.

Section 2.—Duties: The treasurer shall be sole custodian of the cash resources of the Association from whatsoever source obtained. The custodianship shall exclude the revolving fund placed at the disposal of the executive secretary to meet current office expenses, but the treasurer shall hold the executive secretary responsible for the legal disbursement of this fund.

The treasurer shall pay all of the legitimate expenses of the Association, including drafts

for the revolving fund issued by the executive secretary and signed by the president. He shall issue checks against the treasury only on the signed approval of the president and executive secretary, who shall furnish serially numbered vouchers containing full details of the nature of the expenditure. All vouchers and invoices accompanying them shall be filed by the treasurer for a period of not less than five years.

The treasurer shall give a bond to the Executive Board in such amount as the said Board may demand from year to year, and he shall make a detailed report of the financial status of the treasury at each regular annual session or at any other time that the Executive Board may request.

The treasurer shall not be the custodian of the Association's real or personal property but shall receive all income derived therefrom, regardless of its source.

Section 3.—He shall turn over all funds, property and records to his successor.

Section 4.—The repository of the Association's cash resources shall be determined by the Executive Board. For the convenience of making deposits it shall be located in the city where the central office is located. For safety, it shall be a bank of unquestionable financial responsibility.

Article VII

Executive Board Chairman

Section 1.—Election: The Executive Board shall elect a chairman annually at the close of its last meeting held during a regular annual session of the Association. He shall be a duly elected member of the Executive Board. He shall be elected by a majority vote of said Board and shall hold office until his successor shall have been installed.

Section 2.—The election of the Executive Board chairman shall be announced to the House of Representatives at the last meeting of said House held during a regular annual session.

Section 3.—Duties: The chairman shall preside at all meetings of the Executive Board. In his absence from any cause, the Board may elect a temporary chairman.

He shall act as chairman of the Board of Governors, prescribed in section 5, article V of the constitution.

Article VIII

The Executive Board

Section 1.—Number: The Executive Board shall consist of 13 members, as follows: One from each of ten executive board districts, hereinafter designated; a member-at-large; the president of the Association (see paragraph d, section 3, article II of these by-laws); and the president-elect (see section 2, article III of these by-laws).

Section 2.—Tenure:

a) The ten district members and the member-at-large are elected for five years. The member-at-large is elected at a regular annual session at the time and in the manner described in section 1, article III of these by-laws.

b) The ten district members are elected by mail ballot begun at least four months before the term expires, and in the manner hereinafter described.

c) The president and president-elect serve as members of the Executive Board only during their terms of office.

Section 3.—Election: Official ballots are sent to all members of the Association of the district in which a member of the Board is to be elected, with the request to nominate the candidate of their choice on the ballot. The ballots are counted at the end of 60 days. The five nominees receiving the highest number of votes are declared the candidates, whereupon a ballot upon which the names of the five candidates are printed is again sent to all of the members of the district with the request to mark an "X" before the name of their favorite candidate. At the end of a second 60 days, the polls are closed and the ballots counted by a committee of tellers selected from the membership by the executive secretary. The candidate receiving a plurality is declared elected and so notified. The result is published in the JOURNAL and a certificate of election is issued to the successful candidate by the executive secretary.

Section 4.—Duties:

a) The Executive Board, hereinafter called the Board, shall be the administrative body of the Association.

b) It shall be charged with the duty of carrying out the provisions of the constitution and administrative by-laws.

c) The Board shall read a report of all of its actions before each meeting of the House of Representatives, which is authorized to exercise all of the powers delegated to the active members in session that are not in conflict with these by-laws.

d) The Board shall pass on the eligibility of all applicants for membership and upon all charges of misconduct filed against members.

e) A summary of the records of the Board shall be published with the proceedings of the Association annually. The Board, however, may withhold from publication any item that may be deemed prejudicial to the best interests of the Association, and shall have full discretion to withhold from publication any manuscript or transcript of the proceedings of the Association.

f) The Board, through the Board of Governors, shall elect an executive secretary annually in the manner prescribed in section 2, article V of these by-laws.

g) On request of a hundred or more members, the Board may submit any question to the active members for their decision by mail ballot.

h) The Board shall have the accounts of all officers having charge of the funds and property of the Association audited by a qualified accountant, annually, or at such other times as it shall direct.

i) Executive Board Districts: There shall be ten Executive Board districts which shall be identified and constituted as follows:

District I: Dominion of Canada.
District II: Pennsylvania, New Jersey, and Delaware.

District III: Indiana, Illinois, and Wisconsin.
District IV: Alabama, Cuba, District of Columbia, Florida, Georgia, Kentucky, Maryland, Mississippi, North Carolina, Puerto Rico, South America, Tennessee, Virginia, West Indies, and West Virginia.
District V: Iowa and Minnesota.
District VI: Arizona, California, Canal Zone and Central America, Colorado, Mexico, Nevada, New Mexico, and Utah.
District VII: Alaska, Hawaii, Idaho, Montana, Nebraska, North Dakota, Oregon, Washington, Philippine Islands, South Dakota, and Wyoming.
District VIII: Arkansas, Kansas, Louisiana, Missouri, Oklahoma, and Texas.
District IX: Connecticut, Maine, Massachusetts, New Hampshire, New York, Rhode Island and Vermont.
District X: Ohio and Michigan.

Section 5.—The boundaries of the Executive Board districts may be changed or new districts created when changes in the membership are increased or decreased in any one or group of states or territories to the extent of disturbing the equality of representation that the present apportionment is intended to maintain. The number of members in each district shall be approximately the same.

Article IX

House of Representatives

Section 1.—Article VI of the constitution provides for a house of representatives to act for the members in a legislative capacity. This body is hereinafter called the House.

Section 2.—Number: There shall be one member for each component association or group officially affiliated in accordance with article VI of the constitution. He shall be known as the delegate of his association or group.

a) Each component association or official group entitled to a delegate shall choose also an alternate to act in the absence of the delegate.

b) The membership of the House can be enlarged only by the addition of new component associations or official groups.

Section 3.—Election: Delegates to the House may be chosen either by popular vote of their respective associations or by appointment by the executive officers of said associations. Delegates and alternates are chosen in the same manner.

a) The delegate and alternate representing the Army of the United States shall be chosen by the chief of the veterinary division of the Surgeon General's Office.

b) The delegate and alternate representing the Bureau of Animal Industry, U. S. Department of Agriculture, shall be elected or appointed by the National Association of Bureau of Animal Industry Veterinarians.

Section 4.—Tenure: Members of the House are elected for a term of two years, which shall be considered to mean two consecutive annual sessions of the Association. They shall not serve for the third consecutive term unless re-elected or reappointed.

a) The members from the following geographical divisions shall be chosen on even-numbered years:

Alabama	Mississippi	Tennessee
Arkansas	Montana	Utah
Colorado	Nevada	Virginia
Delaware	New Jersey	West Virginia
Florida	New York	Wyoming
Idaho	North Dakota	Canal Zone
Indiana	Oklahoma	Philippines
Kansas	Pennsylvania	Alberta
Louisiana	Pr. Edward Is.	Manitoba
Maryland	Saskatchewan	Nova Scotia
Michigan	South Carolina	

b) The members of the following geographical divisions shall be chosen on odd-numbered years:

Alaska	Maine	Oregon
Arizona	Massachusetts	Puerto Rico
British Colum.	Minnesota	Quebec
California	Missouri	Rhode Island
Connecticut	Nebraska	South Dakota
Dist. of Colum.	New Brunswick	Texas
Georgia	New Hampshire	Vermont
Hawaii	New Mexico	Washington
Illinois	North Carolina	Wisconsin
Iowa	Ohio	
Kentucky	Ontario	

c) The members representing the Army of the United States shall be chosen on odd-numbered years.

d) The members representing the Bureau of Animal Industry, U. S. Department of Agriculture, shall be chosen on even-numbered years.

Section 5.—The voting power of each member of the House shall be regulated by the number of active and corporate members of the Association he represents. Until otherwise determined by action of the Executive Board, the voting power shall be as follows:

50 or less members	1 vote
51 to 150	2 votes
151 to 300	3 votes
301 to 450	4 votes
451 or more	5 votes

a) The voting power shall be determined by the records of the secretary's office at the time the credentials of the delegate and/or alternate were received for filing.

Section 6.—Duties and Authority: The House shall perform all of the duties and exercise all of the authority belonging to the membership except the election of the corporate officers. In principle, the House is the "floor" of the annual sessions.

Section 7.—Credentials: To qualify as a member of the House the delegate and alternate duly chosen by the constituent association or official group shall file promptly a notice of their election on the special form supplied by the executive secretary.

Section 8.—All matters and reports submitted to the House shall be acted upon and referred to the Executive Board. If reported favorably or without comment, the action taken by the Executive Board shall be final.

Section 9.—In event of an irreconcilable dispute between the House of Representatives and the Executive Board, the president is authorized to form a "joint high commission" composed of two members of the House and two

members of the Board, who shall choose a fifth member from the active membership. On questions submitted to it the decisions of the joint high commission shall be final.

Article X

Membership

Section 1.—The classes of members provided in the constitution shall be subject to the following regulations.

Section 2.—Active members, or the general membership, shall be graduates of colleges of veterinary medicine conducted within the United States and the Dominion of Canada which have been declared accredited by a majority vote of the House of Representatives; or, of foreign veterinary colleges approved by the Committee on Education; or, upon recommendations of the Executive Board, of veterinary colleges which have been closed since 1921.

a) *Election to Active Membership.*—Candidates for membership shall present an application to the executive secretary on a special form approved by the Board of Governors. The form shall state the applicant's race, name, age, education, year of graduating and work in which he is engaged. It shall contain the endorsement of two members, one of whom shall live in the same state, province or territory as the applicant.

b) Notice of all applications shall be published in the JOURNAL for two successive months. The first notice shall give the applicant's full name, year of graduation, post office address, and the names of his endorsers. The applicant shall be declared elected and so notified 30 days after the second notice has been published, provided no objection to his election has been filed in writing. Objections to an applicant's election shall be subject to the action of the Executive Board. A rejected applicant may present his defense to the Executive Board through the House of Representatives.

c) *Educational Requirement.*—Excepting the provisions for admitting graduates of non-existing veterinary colleges, described in article X, section 2, only graduates of accredited colleges shall be deemed eligible for membership. The term "accredited college" shall be defined as a regularly constituted veterinary college or division or department of a recognized university or agricultural institution that maintains a course of instruction in veterinary medicine extending over four collegiate years of not less than 32 weeks each and of not less than 17 credit hours per week, and which maintains a matriculation requirement of not less than four years of standard high school work and at least one year of college work leading to a baccalaureate degree, commonly designated as a premedical year. However, accredited colleges may, without prejudice, accept matriculants who, in the absence of the above-named credentials, pass satisfactory examinations before officers of the public educational system approved by the dean.

Section 3.—Dropping Members, Journal Subscriptions, Membership Fee, Dues, Reinstatements:

a) Members who have not paid dues for the current fiscal year, which ends January 1, shall be dropped from the membership but may be restored to the membership list during the following fiscal year by paying the debt owed, provided no charge of other misconduct has been filed against them.

b) Members who have not paid their annual dues on April 1 of the current fiscal year shall be dropped from the subscription list of the JOURNAL, in compliance with postal regulations governing the mailing of second-class matter.

c) Dues shall be $5.00 a year, payable in advance on January 1 of each fiscal year. The dues include payment for the subscription to the JOURNAL and other documents distributed to the membership, such as directories and convention programs.

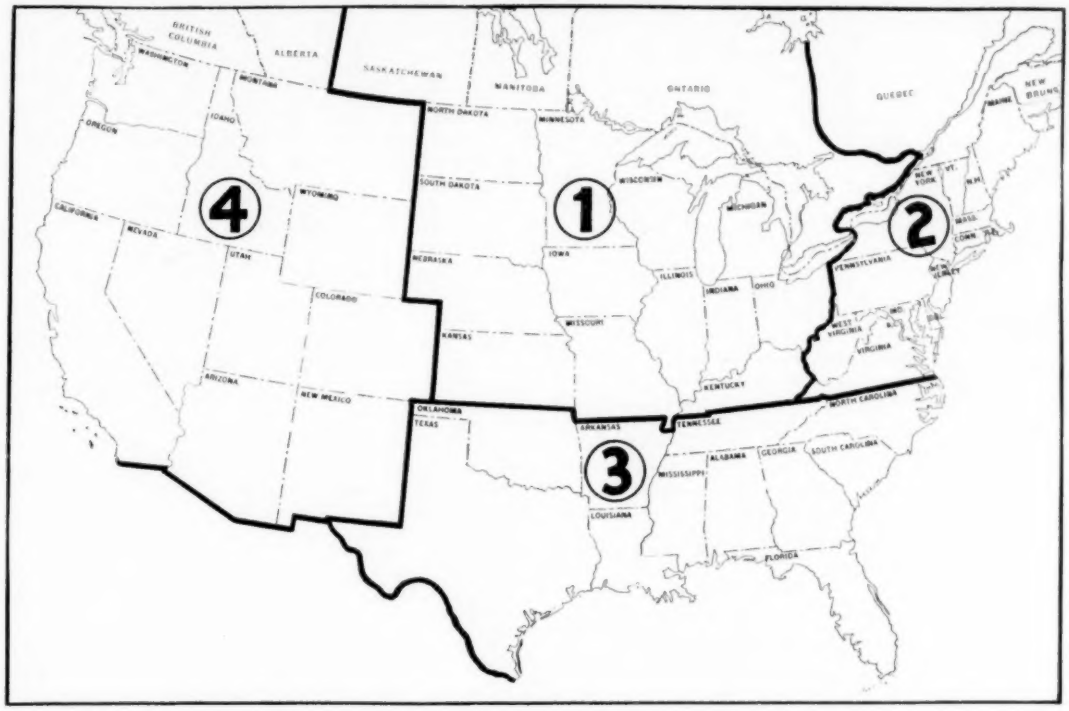
d) *Membership Fee.*—The membership fee is $5.00, payable at the time the application is filed. In order that the annual dues and subscription to the JOURNAL may expire on January 1 of each fiscal year, the following amounts shall be remitted with each application:

January	$10.00	July	$7.50
February	9.58	August	7.08
March	9.16	September	6.67
April	8.75	October	6.25
May	8.33	November	5.83
June	7.91	December	5.42

e) *Dismissals.*—Violation of the code of ethics, conviction of a felony by a court of law, and activities prejudicial to the welfare of the veterinary profession shall constitute ground for dismissal from the membership. The charges shall be filed with the executive secretary in writing and copies of them shall be furnished to the defendant together with a notification to appear before the Executive Board at a stated time and place. In lieu of a personal appearance or appearance by counsel, a written defense shall be acceptable. The publication of charges or transcript of the trial resulting therefrom shall not be published in the JOURNAL unless specifically ordered by a majority vote of the Executive Board.

f) *Reinstatements.*—Members dropped for non-payment of dues may be reinstated by paying their indebtedness to the Association, or by reapplying for membership as provided in section 2 of article X. A member dismissed for cause under paragraph e, section 3, article X, may reapply for membership on the unanimous consent of the Executive Board.

Section 4.—Junior Members: A student of an accredited veterinary college which maintains a junior chapter of the American Veterinary Medical Association may become a junior member by maintaining membership in his local chapter. If reported in good standing by the officers of his chapter, he shall be issued a membership card and such other tokens as the Association may issue from time to time. Junior members are entitled to all of the privileges of active members at the annual session except the right of franchise and shall be furnished an official badge without the payment of the registration fee.



APPORTIONMENT OF AREAS FOR A.V.M.A. CONVENTIONS

Every other year, beginning with 1941, the annual meeting of the Association will be held in area 1. The conventions of the even-numbered years will be held in areas 4, 3, and 2, respectively. Thus, the 1942 meeting will be held in area 4; 1944, area 3; 1946, area 2.

Section 5.—Associate Members: On recommendation of the Executive Board and approval of the House of Representatives, a stated number of associate members may be elected to the membership.

a) The number of associate members shall be determined by the Executive Board and approved by the House of Representatives.

b) Associate members shall be college graduates other than graduates of veterinary colleges who are engaged in research on diseases of domestic animals or wild life in recognized colleges or institutes.

c) They shall be vouched for by the component association of the state in which they live and otherwise comply with the provisions of the constitution and administrative by-laws governing the admission of active members.

d) Associate members shall have the same rights, status, and privileges as active members.

Article XI

Constitutional Sessions

Section 1.—a) Time: An annual session shall be held between July 1 and December 31 of each calendar year. The exact date shall be fixed by the Executive Board at least four months prior to the time decided upon.

b) Place: The United States and the Dominion of Canada shall be divided into four convention zones for the holding of annual sessions in such numerical order as to favor alike all sections within the contiguous territory of these two countries. The selection of the place of holding the annual session shall be governed by the following rotation during the years named and during succeeding years, or until this clause is legally amended:

1940.....Zone 2	1944.....Zone 3
1941.....Zone 1	1945.....Zone 1
1942.....Zone 4	1946.....Zone 2
1943.....Zone 1	1947.....Zone 1

The boundaries of the convention zones are shown in the official map herewith reproduced.

c) Invitations: Invitations for annual sessions shall be filed with the executive secretary not less than two years and four months prior to the date of the session concerned and they shall be presented to the House of Representatives for action two inter-convention years before the meeting is to be held. The city in which the annual session shall be held shall be decided by vote of the House of Representatives.

d) Duration: The duration of annual sessions shall be fixed by the Executive Board.

Section 2.—Quorum: Twenty-five per cent of the number of members registered at an annual session shall constitute a quorum for the transaction of business at a general session.

Section 3.—Order of Business: The order of business shall be as follows:

a) Call to order by the presiding officer;
b) call of the enrollment;
c) presentation of minutes;
d) annual address of the president;
e) presentation of the presidential key to the president-elect;
f) presentation of awards;
g) nomination of officers;
h) presentation of the official scroll to the outgoing president; and
i) adjournment.

Section 4.—No special meeting of the general membership shall be held for the transaction of business except on petition of a majority of the component associations, when an unusual emergency arises. Such meetings and their purpose shall be announced to the entire membership through the JOURNAL, or otherwise, at least 30 days prior to the date fixed for the meeting. Business not thus announced shall not be transacted at a special meeting.

Section 5.—All sessions, including the section meetings, shall be ruled under customary parliamentary usage, as set down in approved "rules of order."

Article XII

Committees—Standing and Special

Section 1.—The president shall appoint the following standing committees for such time and purpose as hereinafter described:

1. COMMITTEE ON BUDGET

a) *Personnel.*—The Committee on Budget shall consist of the president, first vice-president, executive secretary, treasurer, and the chairman of the Executive Board.

b) *Duty.*—Its members shall study the financial affairs of the Association, propose means of increasing the income and provide plans for reducing expenses. It shall set the amount to be appropriated for the officers, committees and special projects legally undertaken by the Association.

c) The executive secretary shall publish the report of the Committee with the business proceedings of the annual session.

d) *Reports.*—The Committee shall report its actions to the Executive Board for consideration and presentation to the House of Representatives.

2. COMMITTEE ON EDUCATION

a) *Personnel.*—This committee shall consist of five members appointed by the president at the rate of one member per year, each to serve for a term of five years. Not less that three members of this committee shall be members of the teaching staffs of veterinary colleges accredited by the Association but no two members shall be of the same faculty or graduates of the same veterinary college.

b) *Duty.*—It shall make an annual report on the status and needs of veterinary education as conducted in the existing veterinary colleges; on the relation of veterinary education to animal production; and on the number of qualified veterinarians required to maintain a competent veterinary service for the American people. When deemed necessary, the committee is authorized to inspect veterinary colleges and submit annually a list of such colleges as should be accredited by the Association.

3. COMMITTEE ON LEGISLATION

a) *Personnel.*—This committee shall consist of five members, four of whom are appointed by the president for a term of five years at the rate of one per year and the executive secretary, who shall act as chairman.

b) *Duty.*—The Committee on Legislation is delegated to watch for subversive legislation in national, state and municipal legislative bodies that is detrimental to the progress of veterinary medicine and to enlighten such bodies on the enactment of constructive measures of interest to veterinary service and the production of domestic animals.

4. COMMITTEE ON PROGRAM

a) *Personnel.*—This committee shall consist of the secretaries of the regular sections and the executive secretary, who shall act as chairman.

b) *Duty.*—It shall compile the program of the annual session, procure suitable contributors and titles for the programs of the respective sections, submit a report of the progress of its work to the chairman in sufficient time to avoid duplications and thereby insure a broad coverage of the field of veterinary medicine.

c) All reporters shall be instructed to register their names and titles with the chairman at least four months before the annual session.

d) The Committee on Program is responsible for all technical exhibits, educational exhibits, demonstrations and clinics in coöperation with the Committee on Local Arrangements. It shall provide reporters and all necessary material required in the presentation of contributions.

5. COMMITTEE ON PUBLIC RELATIONS

a) *Personnel.*—This committee shall be composed of five members appointed by the president for five years at the rate of one per year. The president shall appoint the chairman.

b) *Duty.*—The Committee on Public Relations shall take over the former duties of the Committee on Policy, which is hereby discontinued. Its duties shall be that of studying the relations of the veterinary service to other branches of science and industry and carrying out educational programs beneficial to the general welfare of mankind through the medium of the scientific and public press, civic clubs, professional groups and radio.

6. COMMITTEE ON VETERINARY BIOLOGICAL PRODUCTS

a) *Personnel.*—This committee shall consist of five members, one of whom shall be appointed annually by the president for a term of five years. The first membership shall be appointed for one, two, three, four, and five years, respectively, for terms expiring in the same order. The Committee shall consist of members actively engaged in the field of veterinary biological work. The chairman shall be appointed by the president and approved by the Executive Board. The executive secretary or

assistant executive secretary shall be a member *ex-officio*.

b) *Duties.*—It shall be the duty of the Committee to study the prophylactic and therapeutic merits of the various biological products employed in the practice of veterinary medicine and to present a report of its findings at each annual meeting.

7. COMMITTEE ON PROPRIETARY PHARMACEUTICALS

a) *Personnel.*—This committee shall consist of five members, the majority of whom shall be actively engaged either in teaching or research work on pharmacology and/or therapeutics. The terms of office shall be five years. The president shall appoint members at the rate of one per year. The president shall appoint the chairman. The executive secretary or assistant executive secretary shall be a member *ex-officio*.

b) *Duty.*—The functions of this committee are to study the merits of proprietary pharmaceutical preparations employed in the practice of veterinary medicine; to coöperate with the Food and Drug Administration of the United States Department of Agriculture and the Federal Trade Commission in removing undesirable products from the drug and food market; to coöperate with similar groups in the medical profession engaged in the same activities; and to submit a report of its work at each annual session.

8. COMMITTEE ON RESOLUTIONS

a) *Personnel.*—This committee shall consist of seven members appointed by the president for a term of one year. The president shall designate the member who shall serve as chairman.

b) *Duty.*—To frame, receive and present resolutions for the good of the Association shall be the duty of this committee.

Special Committees

Section 1.—The president shall be empowered to appoint committees delegated to perform special duties. Their term of office shall be one year. The duties, members and chairmanship of special committees shall be designated to the House of Representatives in the announcement of their appointment.

Article XIII

Amendments

Section 1.—The administrative by-laws may be amended at any annual session by the same procedure as that provided for amending the Constitution.

Section 2.—Excepting the sections affecting the corporate officers provided in the constitution, any part of the administrative by-laws may be temporarily suspended by a two-thirds vote of the House of Representatives.

Section 3.—Excepting sections affecting the corporate officers provided in the constitution, the administrative by-laws may be permanently amended at any annual session by submitting, in writing, notice thereof to all the membership 30 days prior to the annual session at which final action is to be taken.

Article XIV

Sections

Section 1.—The Association shall be divided into the following sections:

a) General Practice,
b) Surgery and Obstetrics,
c) Research,
d) Small Animals,
e) Sanitary Science and Food Hygiene, and
f) Poultry.

Section 2.—The officers of each section shall consist of a chairman and a secretary.

a) The officers of the sections shall be chosen by the president from section members nominated by ballot by each section or, in lieu of such nomination, by direct appointment.

b) The secretaries of the several sections shall be members *ex-officio* of the Committee on Program.

Article XV

Registration

Section 1.—The executive secretary shall provide ample facilities for registration and the collection of the registration fee at the regular meetings.

Section 2.—The amount of the registration shall be fixed annually by the Executive Board. The fund collected for registration shall be used in defraying the expenses of the Committee on Local Arrangements.

Section 3.—The executive secretary shall issue badges to the members, ladies, guests, visitors and exhibitors entitled to receive them. No person shall be admitted to meetings or any part of the proceedings until he has registered and been furnished with the official badge.

Article XVI

Property

Section 1.—The executive secretary shall be the custodian of all the real and personal property of the Association.

a) A list of all personal property, such as deeds, securities, leases, insurance policies, charters, furniture and fixtures, machinery and apparatus, books, and other moveable articles, shall be kept available for inspection.

b) A list of the Association's property and its approximate value shall be included in the annual report of the executive secretary.

Article XVII

Publications

Section 1.—The Board of Governors is authorized to publish a monthly periodical which shall be known as the Journal of the American Veterinary Medical Association, and such other printed material as may be deemed advisable in conducting the affairs of the Association.

Section 2.—With the consent of the Board of Governors, the executive secretary or other employé may be designated as the editor of the JOURNAL.

Section 3.—The board of Governors, formerly known as the Committee on JOURNAL, shall serve in the capacity of a managing editor.

Section 4.—Members specializing in the various branches of veterinary medicine may be appointed associate editors.

CLINICAL DATA

Actinobacillosis in Rams*

By C. L. DAVIS, D.V.M.
and GEO. W. STILES
Denver, Colo.

Fig. 1. A portion of the skin of the face, showing several subcutaneous abscesses. Note the needle-grass awns in the wool.

THE RATHER common pyogenic infections in the bovine involving the head and its component parts are well known in veterinary practice. Yet, except for caseous lymphadenitis, these conditions in sheep seldom have been observed. Although both the bone and soft tissue involvements are regarded clinically as being in the same category, the bony suppurations are caused by organisms of the genus Actinomyces (*Actinomyces bovis*) while, in most instances, the cases involving the soft tissue are caused by organisms of the genus Actinobacillus (*Actinobacillus lignieresi*). Despite the fact that the majority of the nonbony cases are found to be actinobacillosis, the writers have seen actinomycosis of the submaxillary salivary gland, tongue, reticulum, mammae, lungs, and peritoneum. In all of these cases there were no associated bone lesions.

Sometimes, both diseases are found in the same animal. The writers had occasion to study such a case in which actinomycosis of the mandible and actinobacillosis of the submaxillary, postpharyngeal and prefemoral lymph nodes were found. To date, both conditions within the same lesion have not been observed by the writers.

Grossly, the purulent contents of these lesions are similar. Actinomycotic pus usually contains sulfur granules that may be seen macroscopically, while in actinobacillosis the granules are in the form of thin, whitish flakes visible only with the aid of a hand lens. Press preparations of the pus in both diseases reveal rosettes, but in our experience these are usually larger in actinomycosis. Smears stained by Gram's method show Gram-positive organisms in actinomycosis but not in actinobacillosis.

The organisms differ materially as to their cultural and morphological characteristics. *A. bovis* grows best under anaerobic conditions and produces small, fine colonies after 48 to 72 hours of incubation. The organism is usually filamentous, but occasionally coccoid forms which resemble streptococci are seen. Some difficulty is usually experienced, however, in recovering the organism from bone lesions. Other organisms, such as staphylococci and *Corynebacterium pyogenes,* are often present, even in closed lesions. On the other hand, *A. lignieresi,* which grows under aerobic conditions, produces colonies within 24 hours which on serum agar resemble the colonies of *Escherichia coli.* The organism is a short, nonmotile, Gram-negative rod (frequently coccoid) which produces acid but no gas in dextrose, galactose, mannite, maltose, saccharose, and levulose. Lactose, raffinose, glycerol and, sometimes, xytose are acidified after extended incubation.

Histologically, the two diseases are granulomatous, starting as suppurative processes which are eventually replaced by

*From the Denver branch pathological laboratory, Bureau of Animal Industry, U. S. Department of Agriculture.

granulation tissue and connective tissue cells. The tissue and cellular reactions are essentially the same and afford no means of exact differentiation. Giant cells usually are seen in both conditions. The rosettes in actinomycosis are inclined to be variable in shape and size and are usually somewhat larger than the uniformly small rosettes observed in actinobacillosis. The organisms in actinomycosis are Gram-positive and in section appear as coccoid or filamentous forms within the rosettes. On the other hand, the organisms in actinobacillosis are Gram-negative and are readily demonstrable within the rosettes as short rods by the Giemsa or eosin-methylene-blue stain. Occasionally, the clubbed ends of some rosettes in cases of actinobacillosis retain the Gram stain and may be mistaken for Gram-positive organisms.

Marsh and Wilkins[1] recently reported actinobacillosis in sheep in this country and Thomas (1931), referred to by these writers, reported the occurrence of the disease in South African sheep as the result of feeding prickly pear. Marsh and Wilkins observed the condition in two different flocks of sheep in Montana. On one farm a large percentage of 2,000 ewes, which had been fed hay during the winter, were affected and about 60 of them died as an indirect result of the disease. In both outbreaks the lesions were confined to the facial region and the cervical lymph nodes.

Hudson[2] recently observed a clinical case of actinomycosis of the jaw in a sheep on the island of Antigua, B. W. I. The affected ewe had an abscess in the jaw which contained a thick, gritty, greenish-yellow pus. Press preparations of the granules in the pus revealed the typical rosette form of the ray fungus.

REPORT OF A CASE

History.—On September 16, 1939, a skin specimen and a cervical lymph node from a ram were received for diagnosis from F. L. Schneider, U. S. bureau of animal industry inspector in charge for New Mexico. C. A. Curtis, who had been detailed to investigate the case, reported that out of a lot of 80 rams on a New Mexico sheep ranch, six showed pronounced swellings about the lower lips, lower portion of the face and neck. Two rams showing the same symptoms died prior to the investigation. There were superficial scabs over the involved areas from which pus could be expressed. Numerous needle-grass awns were seen in the wool and in the skin of the face and legs of all the rams in the lot. The affected sheep showed temperatures ranging from 102° to 106° F., and there

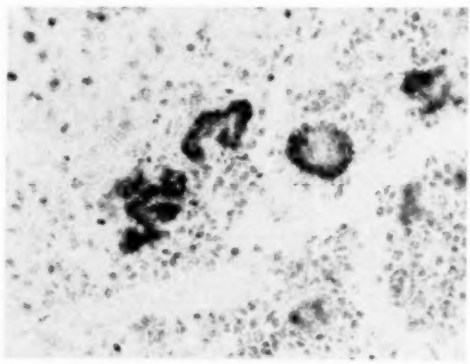


Fig. 2. A section of the lymph node stained by eosin and methylene blue, showing masses of organisms within the rosettes (x 300).

was rapid breathing and heart action. Food could be taken only with difficulty; therefore, lack of sufficient nourishment may have accounted for the deaths. One ram which was dying was destroyed for autopsy purposes.

Autopsy Findings.—The lower lip was greatly thickened and the skin of the lower portion of the face contained several encapsulated subcutaneous abscesses, the largest measuring 40 mm. (1½ in.) in diameter (fig. 1). The tissue between the abscesses showed marked induration. The pus was greenish yellow and viscid and not unlike the pus seen in the lesions of bovine actinobacillosis. The submaxillary lymph nodes also contained lesions of the same character. Several needle-grass awns, buried deep in some of the skin abscesses, were observed.

Microscopic Findings.—Press preparations of the pus from the skin and lymph-node lesions revealed numerous, rather small, ill-defined rosettes. The Gram stain

showed no Gram-positive forms in smears. Cultures on serum-agar slants developed coliform colonies after 24 hours of incubation at 37.5° C. The organisms were short, nonmotile, Gram-negative rods which produced acid but no gas in dextrose, lactose, maltose, saccharose, xylose, and levulose. The cultural and morphological characteristics identified the organism as *A. lignieresi*. Sections prepared from the lymph node showed a granulomatous process containing scattered purulent foci which varied in size. Numerous comparatively small rosettes were seen within the larger purulent foci (fig. 2). Granulation tissue had replaced much of the purulent material. In a few areas connective tissue had replaced the inflammatory cells. Giant cells were not seen in any of the sections. The Gram stain failed to show any Gram-positive bacterial forms. Numerous short rods were seen within the rosettes when stained by both the Giemsa and eosin-methylene-blue stains for bacteria.

DISCUSSION

Although actinobacillosis is a rather common disease in the bovine, the condition appears to be relatively infrequent in sheep. The fact that the disease has been found in Montana and, as reported herein, in New Mexico would lead one to suspect that the malady may exist among sheep in other western states. Swellings about the lips and face, with involvement of the regional lymph nodes, were observed in the affected animals, and the presence of such lesions may suggest actinobacillosis clinically. The superficial scab formations on the swollen lips when observed by Marsh and Wilkins were first thought to resemble the lesions of contagious ecthyma. They failed to demonstrate the virus of sore mouth disease by lamb-inoculation tests. The mode of infection in the New Mexico cases strongly points to mechanical introduction of the disease by penetration of the needle-grass awns through the skin tissues. Thomas associated the disease in South African sheep with the feeding of prickly pear. On the other hand, in one of the Montana outbreaks, which occurred in the spring, the sheep had been fed hay during the winter.

Hay frequently contains foreign material, such as barbed awns of grains and certain grasses, which may injure the mucous membranes and permit infection either through the abraded surfaces or by actual penetration of the barbed awns into the tissues. In tongue lesions in cattle it is not uncommon to find small foreign bodies in the center of the superficial abscesses.

Cultural methods, when possible, are most desirable to differentiate these diseases from other suppurative processes. The presence of rosettes, however, is not always diagnostic for these conditions, since we have seen distinct rosette formations in the pus and in sections from several cases of coccoidioidal granuloma. Histologically the two diseases may be differentiated by the presence or abscence of Gram-positive organisms within the rosettes. Also, the rosettes in actinomycosis are generally more variable in shape and size and are usually larger than in actinobacillosis.

SUMMARY

An outbreak of actinobacillosis in a band of New Mexico rams is reported. Six of a lot of 80 rams were affected. The lesions were confined to the lips and skin of the face and neck, with involvement of the adjacent lymph nodes. The infection was probably introduced by the penetration of needle-grass awns through the skin tissues.

References

[1]Marsh, H. and Wilkins, H. W.: Actinobacillosis in sheep. Jour. A.V.M.A., xciv (1939), n. s. 47 (4), part II, pp. 363-364.

[2]Hudson, L. R.: Actinomycosis in a sheep. Abst. in Vet. Med. (Nov. 1939), p. 681.

The use of pure drugs and a thorough knowledge of just what to do are the bases of internal medicine.

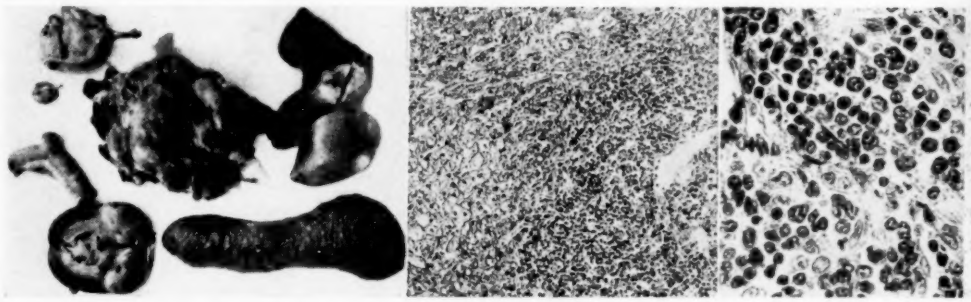
Influenza is the greatest of war scourges. It kills more soldiers than shot and shell and neither a reliable treatment nor a mode of prevention has been discovered.

Two Cases of Malignant Neoplasm in Dogs*

By L. B. SHOLL, B.S., D.V.M., M.S., R. LANGHAM, and E. K. SALES, D.V.M.
East Lansing, Mich.

SARCOMA

The subject was a male Springer Spaniel about 1½ years of age. He had been sick for about three weeks when brought to the clinic. There was anorexia; the feces contained some blood and small pieces of bone; the mucosae were pale and the hair coat rough; there was pronounced dyspnea; the tonsils were enlarged; the temperature was 102.4° F.

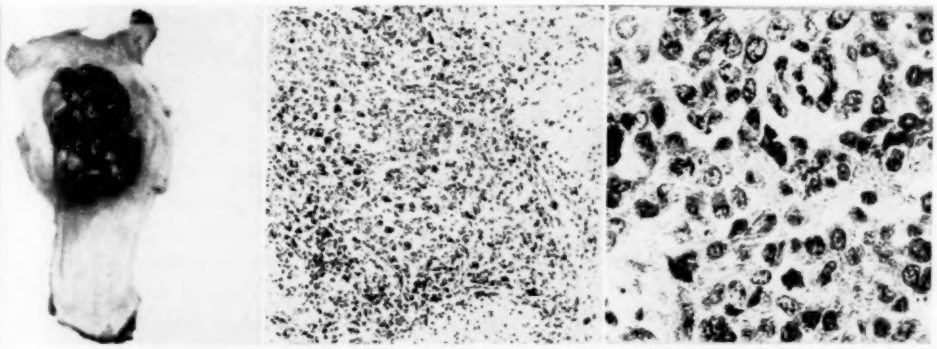


Figs. 1-3 (left to right). 1) The central portion of this figure illustrates a sarcomatous mass involving the prostate gland of the dog. Also shown are metastases to the liver, kidney, spleen, intestine, tonsil and lymph node (x 1/6). 2) Photomicrograph of the liver; right half shows sarcomatous invasion of the organ (x 95). 3) Same specimen as figure 2. Several mitotic figures are seen (x 360).

A blood examination revealed the following:

Hemoglobin	6.5 Gm. per cent (44%)
Red cells	2,420,000 per cmm.
White cells	39,750 per cmm.
Neutrophils	54 per cent
Lymphocytes	39 per cent
Monocytes	7 per cent
Glucose	45.45 mg. per cent
Non-protein N	29.41 mg. per cent
Chlorides	580.00 mg. per cent

An exploratory laparotomy was performed. The condition was noted and the dog was subjected to euthanasia.



Figs. 4-6 (left to right). 4) Osteogenic sarcoma, inner surface of posterior portion of esophagus of the patient (x 2/9). 5) Photomicrograph of specimen illustrated in figure 4. The lighter portion in the upper right corner shows osteoid tissue (x 62). 6) Photomicrograph of specimen illustrated in figure 4, showing sarcomatous portion of the neoplasm. Three mitotic figures are seen (x 360).

Autopsy.—The tonsils were considerably enlarged, apparently being involved by the metastatic neoplasm. The prepectoral and hepatic lymph nodes also showed some neoplastic involvement. Both ventricles of the heart appeared dilated. Several light gray, spherical growths 2 to 4 mm. (.079-.157 in.) in diameter were noted in the spleen. The pancreas showed some congestion. The liver was somewhat yellowish

*From the Michigan Agricultural Experiment Station; published with the approval of the director as journal article No. 366, n. s.

and mottled in appearance, suggestive of fat. Several neoplasms 1 to 3 cm. (.39-1.18 in.) in diameter were scattered through the liver. There was some congestion and excess mucus in the stomach. Some congestion was noted in the small intestine. Four neoplasms 1.5 to 2.5 cm. in diameter were present in the walls of the small intestine. One larger neoplasm was present just anterior to the cecum. In the cortex of the right kidney there was a neoplasm about 3 cm. in diameter. Two smaller neoplasms were noted in the cortex of the left kidney. The urinary bladder was markedly distended with clear urine. In the region normally occupied by the prostate there was a large growth weighing 776 Gm. (26 oz.) and measuring 12 to 14 cm. (4.7-5.5 in.) in diameter. This apparently was the primary neoplasm.

Microscopic Examination.—Sections taken from the primary mass and other organs revealed a highly malignant, connective tissue tumor of the round-cell type (fig. 2). The cells measured 6 to 10 μ (.00023622-.0003970 in.) in diameter and had a small amount of cytoplasm that took the acid stain. The nucleus resembled that of a lymphocyte, but the chromatin granules were slightly smaller. Most of the cells appeared to have a definite nucleolus. They were highly infiltrative and caused atrophy and necrosis in all invaded organs. There was an abundance of mitotic figures and a scarcity of stroma throughout the neoplasm.

OSTEOGENIC SARCOMA

The animal was a large male hound, 8 years old. He was brought to the clinic on May 5, 1938, with the history that he had been noticeably sick since February 18, 1938. He had been examined by three veterinarians, one of whom performed an exploratory laparotomy without finding anything abnormal.

At the time of admission the hound was emaciated and weak; pulse, 148; respiration, 18; temperature, 101.8° F. There was some drooling of saliva and both parotid salivary glands appeared somewhat enlarged. Some coughing was noted. The visible mucosae were anemic.

Fecal examination revealed the presence of hookworms, tapeworms and whipworms. The indicated treatment was given.

X-ray examination revealed an irregular, dense area in the dorso-posterior portion of the chest just anterior to the diaphragm.

A blood examination on May 6 revealed the following:

Hemoglobin	6.9	Gm. per cent (48%)
Red cells	3,750,000	per cmm.
White cells	22,150	per cmm.
Glucose	151.51	mg. per cent
Non-protein N.	27.77	mg. per cent
Creatinine	1.10	mg. per cent
Uric acid	1.90	mg. per cent
Chlorides	550.00	mg. per cent

On May 17, the animal appeared worse. A second blood examination revealed the following:

Hemoglobin	6.1	Gm. per cent (42%)
Red cells	3,450,000	per cmm.
White cells	35,250	per cmm.
Glucose	112.35	mg. per cent
Non-protein N.	30.00	mg. per cent
Creatinine	1.11	mg. per cent
Urea	7.50	mg. per cent
Uric acid	2.00	mg. per cent
Chlorides	500.00	mg. per cent

Urine examination on May 17 revealed no albumin, sugar or acetone bodies. The sediment contained leucocytes, bacteria and epithelial cells.

The x-ray examination, progressive anemia, progressive leucocytosis and emaciation led to a diagnosis of thoracic neoplasm and, with the consent of the owner, the dog was subjected to euthanasia.

Autopsy.—Just anterior to the diaphragm the esophagus was enormously dilated. (See figure 4.) In its wall there was an irregularly spherical growth about 7 cm. (2¾ in.) in diameter. This infiltrated the wall and extended into the lumen as a rather irregular, cauliflower-like growth, on the surface of which there was some inflammation. Some edema was noted in the axillary and prescapular lymph nodes. The parotid salivary glands showed some hypertrophy. There were some fibrous pleural adhesions over the esophagus in the area over the neoplasm. Seven grayish, spherical neoplasms 5 to 10 mm. (.197-.394 in.)

in diameter were present in the lungs, apparently metastases from the esophageal neoplasm. The small intestine contained a few hookworms.

Microscopic Examination. — Microscopic examination showed the growth to be a malignant connective tissue tumor with the general tendency to form osteoid tissue (fig. 5). The embryonic bone tissue showed large numbers of osteoblasts and osteoclasts between which a dense, intercellular substance or osseomucin had formed. In a few areas definite lucunae had formed and the matrix around them showed a slight deposition of calcium salts. The greater portion of the neoplasm consisted of cells spindle, round, and polyhedral in shape (fig. 6). The stroma was scanty, as revealed by special stains. Large numbers of mitotic figures were seen.

The metastatic growths in the lungs showed the same characteristics as described above.

CONCLUSIONS

Both malignant neoplasms gave rise to definite anemia and leucocytosis, apparently quite a common finding in such conditions. It is of interest to note that one appeared in an 8-year-old dog while the other appeared in a 1½-year-old dog.

The Effect of Cereal Grains on Egg Values

Numerous experiments have been made to determine the effect on hatchability and egg production of the various cereal grains available for the feeding of chickens of the average farm flock. Although commercial feeds for chickens are sold on a large scale, it can not be denied that the average farm flock is maintained as a commissary for the home cupboard and is fed mainly from provisions available in the farm granaries. That these majestic aristocrats of the American farm need more than the customary oyster shell to complete the ration of the farm flock is no longer debated in any company. Few farms have milk products to feed to chickens and such supplements as alfalfa and soya beans require processing to make them feedable. The result is that anyone who feeds chickens as a business or sideline must choose between losing money and buying supplements. The proof of this is seen in egg production, hatchable eggs and chick livability.

While the cereal grains of the farm may constitute the bulk of the chicken ration, they have to be scientifically augmented to keep any flock of chickens off the column of liabilities. A glance through the literature shows an accord on the superiority of mixtures of farm grains over single grains, regardless of the supplements used to improve the ration. Where single cereal grains constitute the bulk, corn stands at the top in the order of efficiency. Wheat, however, gives a higher percentage of hatchability than corn (Halpin and Hays, 1934). Obviously, the higher percentage of vitamin E is the responsible factor in wheat.

Crampton, cited by Call and Wilke (1939), gives the relative values in hatchability as follows: Wheat, 114; corn, 109; barley, 100; and oats, 97. Oats, however, improve livability of chicks, and wheat, when fed alone, is apt to cause diarrhea and increase the mortality. Wheat and oats, on the other hand, produce chicks more vigorous than corn when fed to breeding hens.

The Contents of an Egg

Though the proportion of the several components of a hen's egg varies with breed, strain and exogenous factors, it runs within a definite range, as follows:

Shell	6.7 to 11.47%
Yolk	24.39 to 35.92%
White	54.74 to 65.27%

In a study of 413 eggs laid during a year by six Plymouth Rocks, Jull (1924) found the percentages to be: Shell, 10.58; yolk, 30.99; and white, 58.43. In 1901, Simons reported an analysis showing: Shell, 9 to 11; yolk, 29; and white, 60.5 per cent.— *Excerpts from Poultry Science, July 1939.*

The skin is not just an integument. It is an important organ that not only affects the whole body from without but one that receives also a large variety of baffling injuries from within.

How a Practitioner Can Build a Poultry Practice*

By E. S. WEISNER,† B.S., D.V.M., M.S.
East Lansing, Mich.

THERE IS no exact formula to guide the veterinarian who wishes to build a poultry practice. The type of poultry raising in any given locality must be studied and the veterinarian's approach adjusted to its needs.

In general, the financial returns from a poultry practice are not great. Nevertheless, it is a part of the veterinarian's responsibility to be equipped to render a service in this field.

The World's Poultry Congress this summer was advertised as the "Awakening of the Giant of Agriculture." Statistics show that poultry raising, with an annual gross business in the vicinity of one billion dollars, is the second major agricultural industry. It is estimated that there are more than 80 million people in the United States receiving a portion or all of their income from poultry and its products.

There are many reasons for the high mortality in poultry flocks. The wide distribution of flocks, the traffic in live birds, the failure of owners to realize that there is a biological saturation point, the great diversity in management, and the lack of fundamental knowledge of disease and preventive measures have contributed to a staggering annual mortality. It is improbable that any other livestock project could continue with similar losses.

The low value of the individual bird, the rapid development and early reproduction of flocks, and the ease of disposing of dead and diseased birds are apparently fundamental in permitting the industry to continue its development. One other factor that can not be overlooked is the ability of fowl to tolerate the abuses to which they are subjected and yet continue to be productive. It is said that a hen will show no ill-effect from being injected with tetanus toxin sufficient to kill 18 horses.

It is doubtful if any livestock industry has such an atmosphere of mysticism about it as the poultry industry, or if any livestock group is preyed upon as heavily as poultry raisers. For instance, a few years ago a sulfur preparation was placed on the market to control lice on poultry. The product, when put into the drinking water, permeated the pores and interstices of the skin to destroy and control lice. That nature did not provide the skin of poultry with pores did not greatly interfere with the sale of the material. (See the December 1935 issue of *The North American Veterinarian* for many other comparable "treatments" and "cures.") Many of the abuses to which fowl are subjected are attributable to the passive attitude of the veterinarian toward poultry practice and poultry-disease control.

The average poultry raiser is not equipped to become a disease diagnostician. There is no justification for our believing that he is greatly interested or should be taught much other than preventive measures. For weeks following meetings to which diseased birds are brought for autopsy, they are received at the laboratory partially autopsied. Hence, accurate diagnosis is impossible. After the birds have been in transit for several days, wrapped in papers and tightly sealed in containers, anaerobic decomposition quickly destroys the diagnostic lesions which may have been present.

Fortunately, there are sufficient licensed veterinary practitioners who, if educated in poultry-disease diagnosis and control, could replace these pseudo-diagnosticians. With this thought in mind and with the help of several agencies and several local practitioners as nuclei in their areas, we inaugurated continuation schools for veterinarians in poultry diseases.

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†Extension poultry specialist.

*Presented at the 20th annual veterinary conference of the University of Illinois, Urbana, Ill., October 5-6, 1939.

Schools were held during the evening at two-week intervals at some central point in the area from which the men were drawn. The entire evening was devoted to one subject or a group of closely related diseases. Whenever possible, autopsy material was used. This helped to keep the discussion practical and instructive. The first meeting comprised a discussion of the disease situation and a hurried review of seasonal diseases.

The subject matter for the course of instruction was as follows:

1) The poultry-disease situation and seasonal diseases;
2) anatomy and physiology;
3) feeding;
4) bacterial diseases;
5) respiratory diseases;
6) virus diseases;
7) parasitic diseases; and
8) poultry-disease clinics.

During the winter months four separate schools were conducted which were attended by about 60 veterinarians. Those taking the course of instruction were benefited in many ways and, as a result, are now rendering a more complete service in their communities.

Essential Lymphangitis of Horses

THE PAINFUL disease that strikes the hind leg of draft horses on Monday morning or other morning after a day of rest, named lymphangitis or Monday morning fever by the old authors, is as much of an etiological mystery today as it was when Sollylsel wrote *Le Parfait Marechal* in 1664. The coming of modern pathology has shed no light on the nature of this definite nosological entity. But, what is more mysterious than the nature of the disease itself is its almost universal omission by the modern authors. No one who has practiced veterinary medicine among draft horses has failed to encounter the disease with considerable regularity. Sollylsel, Garsault, Nocard, Cadiac, Robinson, Youatt, Percival, Deickerhoff, and Friedberger wrote of it and such teachers of theory and practice of veterinary medicine as Smith (Andrew), McEachran, Liautard, Bell, Law, Cadiot, McKillip, Baker, Stewart and others spoke of its seriousness and common occurrence.

The symptoms, well known to the equine practitioner, are sudden onset, chills, fever, depression, anorexia, accelerated breathing, rapid pulse rate, congested (sometimes icteric) conjunctiva, constipation, lameness and extreme tenderness of the medial face of the tibial region and thigh. Two days of agony is followed by edema of the whole leg which subsides and disappears during the succeeding week. Recurrent attacks are common and each one leaves its trace in the form of a more or less permanent enlargement of the fetlock and cannon which may, and frequently does, develop into a veritable elephantiasis from which there is no relief.

From time to time, though rarely, the subject pops up in current literature, always, however, without clarifying results. Under the title "La Lymphangite essentialle du cheval," Professor Van Wallendael of the veterinary school of Brussels (*Annales de Médecine Vétérinaire*, Dec. 1936) revived the subject in a lengthy article, reviewing the theories of the past, in which he added the allergic theory—the theory of individual sensitization to the heterogenous proteins with, or perhaps without, the influence of microbic toxins in the alimentary canal.

To attribute essential lymphangitis to alimentary toxicosis is sound reasoning in view of what is now known about toxemias of digestive origin. A day of rest for a highly fed horse is indeed a favorable condition for causing a stasis in the digestive reservoirs, where toxic agents mobilize to raid the circulatory system. Urticaria of the horse and dog, certain outbursts of pruritic dermatoses, and acute mastitis associated with a lazy rumen, are examples of exterior diseases of interior origin.

Why a toxemia of this kind should show such a regular predilection for the lymphatic vessels of a hind leg is explainable by the depressed *vis a tergo* of lymphatic circulation at a difficult point—the medial face of the tibial region, which is devoid of the muscular movements to aid in lifting

returning fluids to the large vascular channels above. The afferent lymphatics of the internal face of the tibial region are definitely a favorable place for irritant toxins to congest and work destruction.

In regard to the treatment, the wise equine practitioner, foreseeing recurrent attacks and possible blemishing of the affected leg, sets out promptly and diligently to cut short the acute phase of the disease. Hot baths followed by gentle exercise to speed up the recalcitrant lymphatic circulation are important measures. The aloetic purgation of the old school remains a standard medication. In fact, essential lymphangitis is one of the febrile diseases in which drastic purgation is well tolerated. Diuretic doses of potassium nitrate were formerly regarded as the follow-up panacea. Today, sodium nitrite, because of its alleged detoxifying action on alimentary material, has replaced the potassium salt. Since sodium is edematogenous and calcium dehydrating, the use of calcium gluconate intravenously is worthy of a trial. Van Wallendael (*loc. cit.*), after noting the rapid disappearance of the edema following an intravenous dose of 60 Gm. (2 oz.) of calcium gluconate, abandoned all other medication, even the orthodox purgative of other days. In lieu of calcium gluconate, parathyroid hormone may be employed to produce the desired hypercalcemia.

Since the old plethora theory means nothing in particular and the theory of local infection can not be confirmed, the allergic or toxic theory is entitled to a place in the controversy over this ailment of the horse.

The Ways of Sulfanilamide

THE THERAPEUTIC activity *in vivo* of sulfurated benzene, of which sulfanilamide is an example, is not only antimicrobian but also antiendotoxic. In 1937, Ledaviti and Vaisman[1] demonstrated for the first time the possibility of an antitoxic chemotherapy. Their work opened a new avenue for the treatment of toxicogenic microbian infections.

The work of L and V was conducted on laboratory mice injected intraperitoneally with cultures of gonococci to which mucin was added. In mice that died in 18 to 48 hours, either from gonococcic peritonitis or from endotoxic intoxication, the peritoneal cavity, particularly in the latter event, no longer contained gonoccoci. These had undergone total lysis before death occurred, and therefore opened the way for a study of the action of these chemical agents on bacterial toxins. In a later study,[2] these authors extended their investigations to the endotoxins of other microbian pathogens: meningococci and various pathogenic bacilli of current importance in comparative pathology.

The conclusion reached from these investigations is that the antiendotoxic action of the sulfamides is quite general. That is to say, medicine must now reckon with chemotherapy as well as serotherapy in neutralizing the toxins of bacterial infections.

Foreign Bodies in the Fore Stomachs of Ruminants

The belief that the ingestion of metallic bodies by cows is a casualty due to the accidental presence of foreign objects in the feed is erroneous. From careful observations the author was able to conclude that ruminants are conscious of the presence of foreign bodies in the feed they take into the mouth. The swallowing of them is not due to gluttony or hasty eating but to a desire for them. The ruminant takes a foreign body into the buccal cavity, wags it about and tastes it before swallowing. These observations were made by feeding cows pieces of wire of various lengths and watching the way they are disposed of. (*N. Baboni in Profilassi, July-August, 1937; abstracted in Recueil de Médecine Vétérinaire, cxv, August, 1938.*)

The chirp of the cricket runs two octaves higher than high C of the piano.

[1] Ledaviti, C., and Vaisman, A.: Compte Rendu, Académie des Sciences, ccv (1937), p. 1108.

[2] *Idem:* La chemotherapie antiendotoxique. Annales de l'Institute Pasteur, lxi (Dec., 1938), pp. 635-661.

SURGERY & OBSTETRICS

Collection of Follicular Fluid from the Ovary of the Mare*

By FREDERICK N. ANDREWS, Ph.D., and FRED F. McKENZIE, Ph.D.
Columbia, Mo.

AS A PORTION of a study of the estrual cycle of the mare, it was desirable to obtain the fluids of ovarian follicles of varying sizes at different stages of the cycle.

The position of the ovaries is not constant but in most mares they are located in the sublumbar region of the abdominal cavity ventral to and 8-15 cm. posterior to the kidneys and 5-12 cm. from the median plane. The ovaries can be palpated per rectum and follicles ranging from 1-7.5 cm. in diameter (depending upon the phase of the estrual cycle) can be detected.

Two types of apparatus, as shown in figure 1, were used in this work. The first (1) was designed by Frederick T. Day, M.R.C.V.S., of Cambridge University, England, and consists of two hollow metal tubes, a series of detachable heads of various sizes, and a hypodermic needle. The needle is attached to the inner tube and can be retracted before the apparatus is used. The second instrument (2) was assembled by the investigators and consists of a 15-gauge, 2-inch hypodermic needle, a rubber catheter, and a bottle adapted for aspiration.

When the Day instrument is used, a head of the approximate diameter of the follicle is attached, the needle is retracted, and the instrument inserted into the vagina. The ovary is then grasped through the rectal wall with either the left or right hand and moved to the vaginal wall dorsolaterally to the os uteri. By manipulation of the instrument in the vagina the follicle and the head of the apparatus are brought into close approximation, with the vaginal wall between; the needle is extended, the vaginal and follicular walls pierced, and the fluid collected as it runs from the center hollow tube.

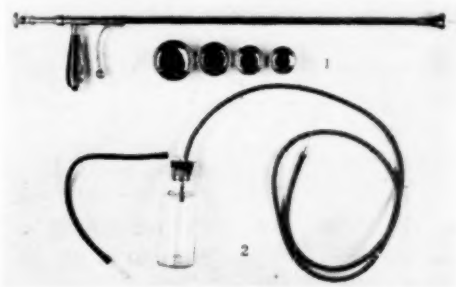

Fig. 1. Types of apparatus used to obtain fluids of ovarian follicles. (See paragraph 3, column 1.)

The Missouri instrument proved to be of considerable value for work on mares difficult to restrain, for its flexibility allowed the animals to move without the needle's becoming withdrawn from the follicle. The order of procedure is usually reversed when the second instrument is used. The ovary is grasped per rectum with the right hand (or left), and the hypodermic needle, covered with a piece of sterile absorbent cotton to prevent laceration of the vaginal wall, is carried into the vagina with the left hand. The ovary is then moved to a position against the vaginal wall as previously described, the cotton removed from the needle, the vaginal and follicular walls pierced, and the fluid aspirated into a bottle. Mares usually show little discomfort during the procedure, but if they do become uneasy and change posi-

*From the department of animal husbandry, Missouri Agricultural Experiment Station, journal series No. 625; Montana Agricultural Experiment Station and U. S. Department of Agriculture coöperating.

tion, the use of the second instrument tends to prevent the disengagement of the needle and the loss of the fluid into the body cavity.

These methods are well adapted for clinical purposes, such as the draining of persistent or cystic follicles, and give the veterinarian information concerning the volume and characteristics of the follicular fluid that might be lost if the follicles are simply ruptured and allowed to drain into the body cavity. Fluids ranging from 6-112 cc. (0.2-3.7 oz.) in volume and containing large amounts of the female sex hormones have been recovered. A detailed report of these findings will soon be published.

Effect of Ovariectomy on Lactating Bitches

By J. E. SEVERIN, D.V.M.
Atlanta, Ga.

CASE 1

A mongrel bitch was brought to the writer for an ovariectomy with a history of having had several litters of puppies. The owner had disposed of the last litter, which was 6 weeks old, on the day prior to presenting the animal for treatment. The writer hesitated to perform the operation, fearing that it might prolong the lactation period, as it does in the cow. This was explained to the owner, but he decided to take this risk in preference to allowing the dog to have more puppies. The operation was performed and, during the seven days in which the wound was healing, the mammary glands took on the appearance of normal, nonfunctioning glands.

CASE 2

A Boston Terrier in whelp to a large mongrel dog was brought to the writer's hospital in fetal dystocia. A laparohysterotomy was advised. The owner requested that an ovariectomy also be performed and that the puppies be destroyed. Just prior to the operation, he requested that one puppy be saved, to prevent any trouble with "caked breast." The operation was successful and the patient and one puppy were discharged. Three days later, the owner reported that it was necessary to feed the puppy artificially, since the milk supply had failed entirely. When the sutures were removed on the seventh day, the mammary glands were in a normal, nonfunctioning condition.

CASE 3

A bitch, heavy in milk, with a history of having just disposed of puppies, was presented for an ovariectomy. The large amount of milk was called to the writer's attention by the owner. The operation was performed, and before the wound had healed, the mammary glands became normal and nonfunctioning.

It appears that there is a difference in the endocrine influence over lactation of the bitch and of the cow in the removal of the ovaries.

Intra-Uterine Medication

Douching the uterus should be done only after the fetal membranes are out. The atonic uterus is unable to expel the water. The water becomes a medium for bacterial growth and the expulsive efforts may injure or rupture the uterus (Williams, 1939).

After the fetal envelopes are out, douching carefully done has value but the fluid used must be gentle in its action. The endometrium is more vulnerable to injury from injected solutions than the bacteria they are intended to destroy. Small amounts are preferable to copious douches and the injected liquid should be syphoned out. Normal salt solution and bland antiseptics suspended in mineral or vegetable oils are used to good advantage.

Animal charcoal in gelatin capsules deposited in the cavity of the empty uterus or retained chorion is sensible intra-uterine therapy. It absorbs harmful elements (bacteria, toxins, gasses) and does not irritate the superficial layer of the endometrium. Mistakes are made in overmedicating the charcoal used for this purpose.

Surgical Intervention in Inguinal Hernia in the Bitch

By CHAS. H. HAASJES, D.V.M.
Shelby, Mich.

THE PURPOSE of this report is to convey the importance of using scissors more frequently than knives in surgery.

The patient was an 8-year-old Beagle, female, with a history of having had several pregnancies. Physical examination disclosed a double inguinal hernia, with large areas of fat covering the hernia.

The surgical field was carefully prepared and anesthetized. A Bard-Parker knife was used merely to incise the skin, precaution being taken not to extend the depth of the incision into any of the underlying tissues. Sharp-pointed scissors were gently forced into the center of the wound and, by means of spreading the blades, the tissues were separated. This procedure was continued through the various layers of fascia, etc.

As blood vessels were exposed, they were ligated at both extremities of the wound. After the hernia sac was brought into the field of operation, it was carefully isolated from the surrounding tissues, its content emptied, and a clamp applied as deeply as possible. This portion of the sac, adjacent to the clamp, was sutured with heavy catgut by means of multiple sutures, leaving two long ends. The sac was then amputated about 1 inch distal to the sutures. The cut edges were then sutured with catgut.

The stump of the sac was returned through the hernial opening into the peritoneal cavity. Care was taken to hold the two long ends of the catgut by means of forceps. One end of the suture was threaded into a round needle, this being inverted and the eye being pushed through the abdominal wall, anterior to the hernial ring. This piece of suture was held by means of a forcep, while the long end around the stump was passed through the abdominal wall in the same way. These were tied in the proper manner.

The purpose of this particular technic is, first, to prevent any possible injury to the abdominal content; second, to prevent the puncturing of any vessel; and, third, to reduce the possibility of recurrence to a minimum.

A large amount of fat was removed and the hernial ring was closed with catgut sutures. The adjacent area, which had been separated by the scissors, was likewise closed with interrupted catgut sutures.

The animal made an uneventful recovery.

Castex

Castex is the proprietary name for a relatively new substitute for plaster of Paris. It is a collodion-like substance contained in bandaging cloth of more compact weave than the cheese cloth used for the frame work of the ordinary plaster bandage. The cloth itself is supporting, application is not messy, and the finished bandage does not interfere with x-ray examinations of the fractured bone, which are so convenient to study the exactitude of the reposition and subsequent course of the healing process. The bandage is resilient. It can be split longitudinally, sprung open, removed, and reapplied without preventing it from serving its purpose.

Castex is available in sealed cans ready for use. The bandage in the can is saturated with acetone. When the can is opened, setting of the bandage begins at once.

Chloral Hydrate

Chloral hydrate is now used so extensively as a somnifacient (= hypnotic) and anesthetic in animal surgery that refreshing the mind on its dynamics is in order.

Chloral, as it is commonly called, is the hydrate of trichloraldehyde obtained by the action of chlorine on alcohol. It was discovered by Lieber in 1832 and was first employed as a somnifacient in 1869 (Sollman).

This drug is the "knock out drops" of the underworld.

The absorption of chloral by the mucous membranes is rapid. In the body it is mainly reduced to trichlorethyl alcohol and, to this reduction, it owes its prompt action. It is excreted with the urine in the form of trichlorethyl-glycuronic acid, which reduces Fehling's solution. This reaction gave rise to the faulty impression that chloral causes glycosuria. The reaction may continue for as long as 18 hours and some authors assert that a hyperglycemia may occur when the anesthesia produced is profound.

Given by the mouth, especially when the stomach is empty, it produces a marked drowsiness in about 20 minutes that will linger for several hours. The respiration and pulse rate are lowered. Experimental evidence has shown that chloral is more prone to depress the heart in animals than in man (Cushny). The lowering of the respiratory and pulse rate is due to its depressive action on the medulla oblongata. Death from overdosing comes from respiratory paralysis and occasionally from cardiac depression. Sudden stoppage of the heart may be caused by relatively small doses (Sollman). This fact, determined by experimental work in cats, is well confirmed in large animal practice, where sudden cardiac collapse occurs during the process of administration. Although this accident is fortunately not common, it is one to be recorded against the use of this alipathic hypnotic in animal surgery. Jung (1914) showed that chloral may kill the fetus *in utero*. Such an outcome is probable if the drug is used upon pregnant animals. Deaths from large doses of chloral may occur also in three to five hours after administration. Resuscitation is best accomplished by hypodermic injections of metrazol and ephedrine.

The safe dose for horses is 30 to 60 Gm. (1 to 2 oz.). By the mouth, it should be given in capsules or well diluted in a watery mucilaginous substance, since in concentration it produces a marked irritation upon the buccal mucosa, especially at the delicate part beneath the tongue. The intravenous dose is about the same, since chloral, being quickly absorbed, exerts its full action no matter how given. This fact must be borne in mind in the use of chloral, for if the intravenous dose is taken as the basis and the dose increased because it is given by the mouth, toxic action may result. Failure of the maximum safe dose to induce the hypnotic effect desired should not be corrected by the administration of additional amounts. In that event the anesthesia should be completed with ether by inhalation.

Solutions for intravenous use are made with normal salt solution, 30 cc. to 480 cc. (1 oz. to the pint).

The popularity of the intravenous use of chloral in horses appears to be due to a strange fear of inhaled anesthetics and the difficulty of administration as compared with the facile intrajugular injection. The objections to its use are the occasional deaths from cardiac collapse and the duration of the narcotic state. A precious hour or two may be lost in waiting for the subject to stand on its feet. Moreover, the sweeping movements of futile struggles to rise may damage the surgeon's wound.

Silk Sutures vs. Catgut Sutures

An editorial in the *Journal of the American Medical Association*, entitled "The Renaissance of Silk in Surgery," is a plea for a return to the custom of 20 years ago in the matter of selecting suture material. Halstead's article against the use of catgut, written in 1913, is found to be full of wisdom by the surgeons of the present day, many of whom are going back to silk for obvious reasons, even for buried sutures.

Catgut is a contaminating medium. Even though sterile, it plays the rôle of necrotic tissue in furnishing culture media for saprophytes. The sensitivity to catgut protein in man and animals is pointed out. This allergic reaction is accountable for the disruption of wounds. Kraissl and associates were able to sensitize guinea pigs to chromic catgut that resulted in the animals' disrupting 30 per cent of the test wounds. With silk, hemostasia is better, irritation is minimal, knots are smaller, and the handling of tissues is more gentle.

CURRENT LITERATURE

ABSTRACTS

Avian Tuberculin in Johne's Disease

The therapeutic value of avian tuberculin in Johne's disease has long been debated. Chazeau and Moussu (1929) reported success with paratubercle antigen. The close relationship of *Mycobacterium paratuberculosis* and *M. tuberculosis avium* is shown by the reaction to avian tuberculin of cattle affected with Johne's disease, but no experimental work has ever been done to determine its therapeutic value.

Five cows and two heifers, all known to be reactors, and one cow classed as suspicious were used. Eight weekly injections of avian tuberculin (5 cc.) subcutaneously were followed after an interval of 90 days with an intravenous injection. After another interval the animals were given a second test and a second series of treatments. The conclusions drawn were that the treatment had no appreciable effect on the course of the disease and that animals affected with Johne's disease are frequently negative to the avian tuberculin test. (*The Cornell Veterinarian, xxix, October 1939, pp. 359-362.*)

Cyanide Poisoning in Hogs

It is commonly known that swine are less vulnerable to HCN than other farm animals, although not entirely immune. To establish the facts in this regard, Couch and Bunyea made a series of tests on the toxicity of potassium cyanide with a group of 81 hogs at the experiment station of the bureau of animal industry at Beltsville, Md., using KCN of reagent quality, and administering various doses by the mouth. Except that the action was slower, the symptoms produced were comparable to those of cyanide poisoning in other animals: Staggering, falling, respiratory distress, coma, paralysis and death. Vomiting occurred in 59 per cent of the cases, and the emesis was found to be a factor in the dose hogs can tolerate. Obviously, vomiting is one of the reasons why cyanides are less fatal for swine.

The weight of the hogs ranged from 77.1 kg. (169.6 lbs.) to 351.5 kg. (773.3 lbs.) and the dose given varied from 0.27 to 7 mg. (0.1 gr.) per kg. (2.2 lbs.). Symptoms appeared in 1.25 to 47 minutes after administration and death in 20 to 120 minutes in the fatal doses. The minimum lethal dose ranged from 2.4 to 3.4 mg. (.036 to .051 gr.) per kg. in the animals that did not vomit. The test showed that hogs are not less tolerant to cyanides (in the form of KCN) than other animals. (*James F. Couch and Hubert Bunyea. Toxicity of Potassium Cyanide for Swine. Veterinary Medicine, xxxiv, October 1939, pp. 620-623.*)

Chaulmoogra Oil as a Microbicide

Tests of the microbicide action against *Mycobacterium tuberculosis avium* in the basic culture media used in producing avian tuberculin showed that no surface growth occurred in any of the six flasks containing chaulmoogra oil in a dilution of 1:100, but acid-fast organisms were found in stains made of all of the flasks. At 1:500 abundant growth occurred in four of the flasks, slight surface growth in one and no surface growth in the other. At 1:1,000 four flasks showed abundant surface growth, one slight surface growth, and one contained a mould. At higher concentrations abundant growth occurred in all of the flasks. The conclusion drawn is that chaulmoogra oil in high dilution has no apparent effect on the growth of that organism. (*The Cornell Veterinarian, xxix, October 1939, pp. 357-359.*)

Accidents on the Farm

Farming is an exceedingly hazardous occupation. The dangers of farming are not generally appreciated. In one study of 310 accidents among rural people, 18 per cent were found to be caused by tools or implements, 16.5 per cent by animals, 12.9 per cent by machinery, 11.6 per cent by vehicles, 5.5 per cent by falling trees, and 2.2 per cent by exposure to heat or cold. Barb wire, nails, splinters, thorns, hooks, chains and firearms come into the figures with a minor share of the causative factors.

Accidents are most common between the ages of 15 and 24 years, and males are injured twice as frequently as females. Of 864 recorded injuries, 289 were fractures, of which 33.7 per cent were compound. The fatalities numbered 16.

An unexpected part of the report is that 20 per cent were not able to pay any part of the cost of professional care and hospitalization, 18 per cent paid a part, and 62 per cent paid in full. The hospital where these statistics were compiled took a loss of $10,000 from this group of patients. *(John H. Cooper, M.D. The Hazards of Farming. Journal of the American Medical Association, cxii, October 7, 1939, pp. 1375-1379.)*

Immunity Experiments on Guinea Pigs

Each of five vaccines, four of virulent tubercle bacilli killed by heat (60° and 100° C.), formalin, and glycerin, respectively, and one of BCG, raised the resistance of the majority of guinea pigs to infection with virulent bovine bacilli. The increase in resistance was not of a high order but was effective in diminishing the extent and severity of the generalized tuberculosis in 50 per cent of the guinea pigs. *(Immunity Experiments on Guinea Pigs with Killed Tubercle Bacilli and BCG. A. S. Griffith and R. E. Glover. Jour. Comp. Path. & Ther., liii, 1939, p. 57.)*

Blood-Type Shock

Out of 21 blood transfusions in horses, the blood of four of the recipients was observed to be incompatible with that of the donors. The incompatibility was signalized by trembling, sweating, lowered pulse and respiration, muscular weakness, urinations, and wobbling of the hind quarters. It was further confirmed by the usual agglutination test; that is, the blood serum of the four recipients agglutinated the blood cells of the respective donors.

The clinical physiognomy after transfusion where there is no incompatibility is little modified. There is a slight increase in pulse rate and blood pressure lasting three to six hours. The temperature rises a point or two but there is no change in the visible mucous membranes. *(J. Koch. Abstract in Recueil de Médecine Vétérinaire, July 1939.)*

BOOK NOTICES

Correction: The price of Edelmann's *Meat Hygiene*, reviewed in the October 1939 issue of the JOURNAL, was given as $5.00. The correct price is $5.50.

Brucellosis in Man and Animals

Diseases caused by organisms of the genus Brucella have attracted such worldwide attention in recent years that nothing less than having the sum of knowledge of them is expected of the veterinarians of this period. Sketchy knowledge of their clinical manifestations in the domestic species is not acceptable as the background of our pledge to safeguard the nation against such grave interchangeable infections. On this account, the revised edition of a book such as Huddleson has just published is a welcome guest. *Brucellosis in Man and Animals* (1939), the successor of *Brucella Infections in Animals and Man* (1934), covers the ground in a manner adequate to satisfy all groups engaged in the study of this threat to human and animal health.

The investigating scientist, the teacher of human and of animal pathology, the livestock sanitarian, the public health officer, the dairy inspector, and the practicing physician and veterinarian will find Huddleson's new book a precious documentation of one of the liveliest problems confronting

medical science today. It is a well-written book, full of scientific and human interest, and devoid of the drab surpluses a writer like Huddleson could have injected *ad infinitum* to the confusion of the reader outside of the research field. We say this because no American veterinary author has been more widely quoted in foreign language literature. The reason for this lies in the wealth of his original work on the more intricate phases of brucelloses.

It is not the province of this brief to analyze the text so plainly ear-marked as an authentic treatment of the subject within the scope of available knowledge. The original work of this well-known author coupled with many citations from an extensive bibliography is ample assurance that the expressed intention to place the last known word on the subject between the covers of a handy manual has been fulfilled.

This work is not a sudden flare from a research laboratory. It is the culmination of eight years of investigational work at the Central Brucella Station of Michigan State College and, although not even hinted in the preface, at many parts of the world visited by the author in his quest of verifying facts. Moreover, such prominent workers on brucellosis as A. V. Hardy of Iowa, J. E. Debono of Malta, and Ward Giltner of Michigan were drafted to present the aspects of the disease for which they have won distinction.

Besides a classical foreword by Giltner and a revealing preface by the author, the book is divided into seven chapters, namely: 1) The Genus Brucella; 2) Methods of Isolating Brucella; 3) Differentiation of the Species of the Genus Brucella; 4) Brucellosis in Human Beings; 5) Brucellosis in Animals; 6) Laboratory Diagnosis of Brucelloses; and 7) Eradication or Control of Sources of Brucellosis Infection. The latter is credited to Ward Giltner. An appendix of 26 reports of human cases in Iowa by Hardy, and a bibliography of 378 references, together with indexes of subjects and authors, complete this highly informative book. *(Brucellosis in Man and Animals. By I. Forest Huddleson, D.V.M., M.S., Ph.D., research professor in bacteriology, Michigan State College. The Commonwealth Fund, New York. Humphrey Milford. Oxford University Press, London. 339 pages, with 40 illustrations. 1939.)*

Diseases of the Genital Organs of Animals

Professor Emeritus W. L. Williams of Cornell University brings out the second edition of the only book on veterinary medicine that has been translated into foreign languages: German and Italian.

The book is not an easy one to review. The author sets down the experiences of a long life in the clinical field which, in many places, abruptly contradict the consense. Furthermore, the book forthwith takes the reader into abstruse studies of embryology, teratology and genital physiology, stretching beyond the average ken. Throughout it expounds the logical idea that an understanding of breeding troubles must be based upon the disorders which overtake mammalian development from the formation of the germ cells to the end of adult life. Although not a few of such disorders are hidden in the mysteries of reproductive physiology, the author makes a creditable effort to syncretize them with the common observations of clinical medicine. In fact, one does not read far into the text without becoming aware of the mysteries of reproduction—of the relation of hidden causes to the troubles of the breeder, the doctor, and the obstetrician. Conspicuous is the good case the author builds up in proving that the study of breeding troubles does not begin or end in the bacteriological laboratory.

In short, Williams wants his readers to consider factors other than the ever-ready microbe. For example, contagious abortion is not a "female complaint." Faulty spermatogenesis of nonmicrobic origin may be the starting point of that nosologic entity as well as the cause of sterility, feeble offspring, retained after-birth and subnormal adults.

When the reader is reconciled to that philosophy, he may wade through the seven sections and many chapters to excellent ad-

vantage. The sarcastic humor habitually used for clinching conclusions is beautifully submerged in the soundness of the arguments. Only strangers would point it out as a *faux pas* in classical literature.

In the chapter on feeds, one is reminded, not without reason, that the whole problem of nutrition is "befogged with dangerous propaganda." Minerals are believed to be abundant in feed and, in part, discharged unused from the body. Bone diseases of horses are not due to a defective supply of calcium phosphate but to a wastage of it, the author declares, and the need of animals for a given food ingredient is "grossly exaggerated." Even in the historic osteomalacia of South African cattle signalized by Theiler, the good result obtained from feeding bone meal is not regarded as proof that the trouble was "wholly due to phosphorus deficiency." "Juggling with vitamins" is justified only where dogs are fed parsnips and cows fed fish. Vitamins, drugs, bacterins, vaccines and intra-uterine therapy can not "prevent the death of the fertilized ovum doomed because of a hereditary lethal defect or imprisonment in an unhealthy uterus."

The author's sense of humor reaches its climax in the chapter on artificial insemination. Here is cited the well-known shipment* of bull semen 5,000 to 7,000 miles (United States to the Argentine) and its alleged arrival in active condition. Since neither the origin nor the destination of the semen are of record and no one has boasted that the purpose of the shipment was achieved, to the author the transaction seems like fiction. Refrigeration or long-time preservation of semen is pronounced a questionable practice and the resort to insemination to prolong the usefulness of incapacitated bulls has been overrated. He cites a case where 30 inseminations from a bull affected with arthritis resulted in one calf of dubious value, and no official report of the incident ever appeared or is contemplated because "it might detract from the value of artificial insemination from crippled bulls." The proper time, place and procedure of artificial insemination are, however, described in revealing details.

The chapter on tumors describes the newgrowths attached to the reproductive organs. Though less common than their respective counterparts in human beings, newgrowths of the ovary, uterus, cervix, vagina, vulva and penis are a class of morbid entities encountered in the course of veterinary practice. However, the short span of life of animals keeps down the occurrence of malignant growths, and benign growths are likewise relatively rare. Dermoid cysts of the gonads, ovarian cysts (other than degeneration of the follicles of Graaf), occasional fibroids of the uterus, and epithelial tumors of the penis are described. Here, a classical technic of amputation of the penis of horses is given with fine illustrative figures.

Section 4 points out the high rate of abortion from generalized infections. When plagues rage, abortions run high. Even infections of low virulence imperil the fetus.

The section entitled "Infections Believed to Have an Affinity for the Reproductive Organs" contains 88 pages, of which 50 are expended on contagious (infectious) abortion which, to Williams, does not mean Bang's disease exclusively. There are other bacillary, virus and protozoan pathogens that are abortifacient. Faith in the ubiquitous bacillus of Bang has submerged them.

The quarantine and slaughter method of controlling Bang's disease comes in for a drubbing. This and other remedies have left the breeders "as happy as the Children of Israel after 40 years in the wilderness." Public and private functionaries are scolded for not publishing authentic and comprehensive reports on the efficiency of the treated herds. The fact that a cow becomes pregnant after treatment does not prove that she has become a profitable cow. The chapter on organic diseases contains a wealth of practical information. If some of it runs contrary to popular beliefs, there is no lack of the rigid conservatism that

*The shipment was made from Beltsville, Md., to Buenos Aires. The distance was 13,400 kilometers and the time was 168 hours. The insemination resulted in the birth of a calf, according to a published report (*The Veterinary Record*, Aug. 19, 1939).

makes safe and useful practitioners of veterinary medicine.

The last section, which deals with troubles of the newborns, will be a disappointment for the reader who is looking for a recipe for calf scours. But, in emphasizing the great changes a fetus undergoes at the birthline, where virtually all functions are suddenly readjusted, the author again shows the importance of coupling fundamental knowledge with common sense.

What of colostrum? The treatment of that subject astonishes. It does not agree with the general, age-old belief that colostrum has laxative and antigenic properties. The preventive treatment of scours is purely hygienic; perhaps killing the bull may be a sensible prophylactic measure. Pyemic arthritis and navel ill "defy classification," notwithstanding that apparently capable authorities set them down as congenital diseases on the ground that they respond to injections of maternal blood.

In what category shall one classify *Diseases of the Genital Organs of Animals?* To quote the author's own words, used for another purpose, it "defies definite classification." Among books on that subject it stands severely alone because through the years the author left the orthodoxies of the past to chart a sea of unknowns where future generations may find smoother sailing. The library of the veterinarian, breeder, herdsman or any one interested in mammalian reproduction that does not contain this worthy volume has a gap to fill up. (*Diseases of the Genital Organs of Animals. By W. L. Williams, professor emeritus, Cornell University. 617 pages, illustrated. Published by the author, 209 White Park Road, Ithaca, N. Y. 1939. Price, $9.00.)*

Air Raids Precautions for Animals

Air Raids Precautions for Animals is the title of a book published by His Majesty's stationery office, London; price, 3d. Pursuant to its publication the National Air Raids Precautions for Animals Committee was formed to carry out its provisions. In the London metropolitan area there are 73,000 head of farm animals and approximately 2,000,000 dogs and cats which will come under the planned protection. In all of England there are 37,500,000 farm animals, 56,250,000 domestic fowl, and between 6,000,000 and 7,000,000 dogs and cats. "If, with London as a basis," says *The Veterinary Record*, "a workable scheme for the protection and care of animals within its boundaries can be evolved, modified schemes to meet the requirements of other cities and towns can be framed." Though sponsored by the national humane society, the veterinary profession will be in the front line of this movement, the editor promises.

"A Guide to Horse Buyers"

"A Guide to Horse Buyers" (Extension Bulletin 197) by R. S. Huston of Michigan State College is an excellent documentation of the subject that will please any horse-minded veterinarian so far as conformations, determination of age, and major blemishes are concerned. But, it will not please the modernist who knows that poll evil and fistula of the withers have long since been removed from the list of traumatisms.

State College of Washington Offers Historical Books on Veterinary Medicine

While in Spain the past summer, J. Horace Nunemaker, head of the foreign language department of the State College of Washington, Pullman, Wash., purchased a number of 16th and 17th century books on veterinary medicine for the College library.

Those who are interested in the history of veterinary medicine will find valuable reading in these books. Information concerning them may be secured from William W. Foote, librarian of the College, or from Dr. Nunemaker.

The practice of lighting up the chicken coop at night to increase egg production was practiced in Spain in the 18th century. A book on the subject was published in Madrid in 1803.

THE NEWS

Student Enrollment Shows Gain for Sixth Consecutive Year

Student enrollment for the academic year 1939-40.

School	Fresh.	Soph.	Jun.	Sen.	Spec.*	Grad.	Total	1938–39	Change
Alabama Polytechnic Institute	77	66	51	49	13	3	259	198	+ 61
Colorado State College	39	43	34	36	0	0	152	144	+ 8
Cornell University	40	43	38	43	1	16	181	169	+ 12
Iowa State College	64	60	62	56	3	7	252	238	+ 14
Kansas State College	61	49	62	58	0	0	230	241	— 11
Michigan State College	61	65	46	61	3	2	238	221	+ 17
Montréal, Université de	13	12	12	13	0	0	50	53	— 3
Ohio State University	65	58	54	47	0	6	230	236	— 6
Ontario Veterinary College	58	71	58	60	0	0	247	259	— 12
Pennsylvania, University of	53	45	45	38	7	0	188	196	— 8
Texas A. & M. College	99	82	76	37	2	0	296	291	+ 5
Washington, State College of	40	41	41	27	0	0	149	140	+ 9
Totals (1939–40)	670	635	579	525	29	34	2,472	2,386	+ 86
Totals (1938–39)	680	617	527	515	10	37	2,386	2,223†	+163

*Figures do not include pre-veterinary students.
†Total number of students for 1937-38.

Again this year, as for each of five academic years past, student enrollment in the recognized veterinary colleges of the United States and Canada shows an upward trend. The figures in the table above reveal for 1939-40 an increment of 86 over the preceding college year. In the following tabulation the regular enrollment (freshmen, sophomores, juniors and seniors) for the decade 1930-31 to 1939-40 is given. It will be observed that in only one instance in this period, 1933-34, was there a decline from the previous figure. For obvious reasons, pre-veterinary students are not considered in either of the tables.

Enrollment of regular students for the ten-year period 1930-31 to 1939-40.

Academic Year	Enrollment
1930–31	1,212
1931–32	1,378
1932–33	1,401
1933–34	1,370
1934–35	1,568
1935–36	1,810
1936–37	1,927
1937–38	2,170
1938–39	2,339
1939–40	2,409

In comparing the senior enrollment for 1938-39 with the number graduated in 1939, one finds that of a total of 515, 506 were graduated. Alabama Polytechnic Institute graduated 28; Colorado State College, 26; Cornell University, 39; Iowa State College, 48; Kansas State College, 68; Michigan State College, 36; Université de Montréal, 13; Ohio State University, 49; Ontario Veterinary College, 62; University of Pennsylvania, 54; Texas A. & M. College, 54; and State College of Washington, 29.

In 1930, the veterinarians of the United States numbered 11,863. Ten years previous there were 13,495. In 1910, the total was 11,652, fewer by over 200 than in the automotive year 1930.

The sixteenth decennial census, to be taken in 1940, will include a careful count of active veterinarians throughout the United States. Enumerations also will be made of packing houses and their activities, canneries, and wholesale and retail outlets for live stock, meat, and farm products.

The U. S. Department of Agriculture has forecast a record turkey crop of 31,957,000 head this year.

U. S. GOVERNMENT

All Herds of Cattle in U. S. Now Tested at Least Once for Tuberculosis

The federal bureau of animal industry announced on November 22 that all herds of cattle in the United States now have been given at least one test for tuberculosis. Testing the last herd signifies that all serious opposition which has hampered official tuberculin testing in various parts of the country has been overcome. Although much retesting still remains to be done, the barriers which threatened the successful completion of the campaign, begun in 1917, are now broken down.

A herd in Stanislaus county, Calif., was the last to receive the initial test. Of 123 cattle in this herd, 95 were free of the disease. The first herd to be officially accredited in the coöperative federal-state campaign against tuberculosis was that of the U. S. soldier's home in Washington, D. C.

In recent months the principal zone of tuberculosis-eradication activity was a group of six counties in central California.

New B. A. I. Order Revises Regulations on Imported Animal By-Products

Though foot-and-mouth disease, rinderpest and anthrax are distinct contagious diseases of animals, former protective regulations designed to bar their entry in the United States grouped them under similar regulations. A recent order issued by the federal bureau of animal industry and applying mainly to hides, skins and other animal by-products deletes anthrax from this grouping and relaxes import restrictions where this disease is the only hazard. But, the order regulates even more strictly certain imports from countries where foot-and-mouth disease and/or rinderpest exist(s).

The modification is the result of investigations which indicate that modern methods for the control of anthrax have made the disease less threatening than formerly and that the rigid restrictions which have prevailed on international trade are no longer necessary. On the other hand, the occurrence of foot-and-mouth disease in new territory abroad makes obvious the need for rigid measures to block its spread to American borders.

Therefore, the new order (No. 371, superseding No. 341, effective since 1933) relaxes import restrictions on animal by-products from countries where anthrax is the principal hazard and imposes more strict regulations on such products where foot-and-mouth disease and rinderpest are known to occur.

Motion Picture Prepared by U. S. D. A. Depicts Eradication of Fowl Tuberculosis

How a farmer eradicated tuberculosis from his poultry and swine is shown in a motion picture produced by the U. S. Department of Agriculture. The film portrays the close relation between tuberculosis of poultry and swine as contrasted with the bovine type, and illustrates the difficulties that are encountered when hygienic principles are not adhered to in the management of the poultry flock.

A coöperative product of the bureau of animal industry and motion-picture technicians of the extension service, the film is available in both 16- and 35-mm. (0.6- and 1.4-in.) widths and requires approximately 23 minutes for projection.

B. A. I. Transfers

Richard E. Baer from Oklahoma City, Okla., to Albuquerque, N. Mex., on Bang's disease; George Berger from Richmond, Va., to Raleigh, N. C., on Bang's disease; Hugh J. Clary from S. St. Paul, Minn., to Billings, Mont., in charge of meat inspection; John H. Collins from East St. Louis, Ill., to Little Rock, Ark., on Bang's disease; Bernard Goldis from Richmond, Va., to Raleigh, N. C., on Bang's disease; Sidney L. Kaplan from Richmond, Va., to New York, N. Y., on meat inspection; Robert L. Knudson from Chicago, Ill., to Beltsville, Md. (Animal Disease Station); Lee Roy Mims from San Juan, P. R., to Jackson, Miss., on Bang's disease; Mark H. Toroian from Richmond, Va., to S. St. Paul, Minn., on meat inspection; Clifford W. Wilder from Richmond, Va., to Raleigh, N. C., on Bang's disease.

Veterinary Corps Reserve

NEW ACCEPTANCES—FIRST LIEUTENANTS

Charles W. Weinberg, 532 Potomac Ave., Buffalo, N. Y.

Glenn A. Zinn, Mindenmines, Mo.

PROMOTIONS—TO CAPTAIN

John S. Bixby, 531 S. Holmes St., Memphis, Tenn.

John M. Holmes, RFD, Chesterland, Ohio.

Joseph B. Morse, 3514 Reisterstown Road, Baltimore, Md.

Edward C. Phipps, 202 E. College, Mayfield, Ky.

AMONG THE STATES

California

The International Association of Milk Dealers met in San Francisco, October 26-28, 1939. Among the veterinarians on the program were C. M. Haring, C. L. Roadhouse, Hugh S. Cameron, O. W. Schalm, J. G. Townsend, and G. H. Hart. Mastitis, Bang's disease and acetonemia were among the diseases discussed.

Delaware

Rabies quarantine has been imposed on New Castle county following an extensive outbreak of the disease in that county during September and October.

District of Columbia

The District of Columbia Veterinary Medical Association held its fourth quarterly meeting of the year at the Mayflower Hotel, Washington, D. C., on the evening of October 19, H. W. Schoening presiding.

E. W. Price, assistant chief of the zoölogical division, U. S. bureau of animal industry, spoke on "The Biological Basis for Control of Parasites in Small Animals." Following, a report on the discussions carried on in the House of Representatives at the Memphis meeting of the A.V.M.A. was given by Mason Weadon, delegate to the House from the District of Columbia. A showing of motion pictures on reproductive disturbances in horses concluded the program.

W. M. MOHLER, *Secretary.*

Idaho

Edwin T. Powell, director of the Idaho bureau of animal industry, recently notified Idaho veterinarians that a new state veterinary laboratory is available for diagnostic work and Bang's disease control. H. Propp is in charge of the Bang's disease laboratory and M. P. Chapman supervises pathology and bacteriology.

Dr. Powell stated that 770 cases of equine encephalomyelitis have been reported during the present year among the state's horse population of 69,000. Thus far, only 90 horses have died of the disease.

Illinois

A law was passed by the Illinois legislature that prohibits vendors of milk from using the term "Grade A" on any milk or dairy product that is not produced and handled under sanitary conditions set forth in the law. Enforcement is in charge of the state health department, which has drawn up a set of regulations for the guidance of all municipalities that have not adopted the model milk ordinance of the U. S. Public Health Service.

Another law prohibits the use of the word "pasteurized" by milk dealers who do not operate in compliance with the standards for that process set down by the state.

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The 35th annual meeting of the Mississippi Valley Veterinary Medical Association, held in Galesburg on November 15-16, was the most successful meeting in the organization's history. Prominent veterinarians from six states contributed to the program. Among the out-of-state speakers were F. C. Tucker of Claypool, Ind.; W. E. Peterson of the University of Minnesota, St. Paul, Minn.; Frank M. Wilson of Mechanicsville, Iowa; G. N. Haigler of St. Louis, Mo.; and Frank Breed of Lincoln, Neb.

Illinois veterinarians who presented papers at the meeting included W. C. Glenney of Elgin; W. D. Daugherty of Sterling; A. S. Robertson of Peoria; L. D. Frederick of Chicago; L. A. Dykstra of Lena; and J. A. Henderson of the University of Illinois, Urbana.

A. R. Cowser of Farmington was elected president; C. M. Rodgers of Avon, vice-president; and L. A. Gray of Bushnell, secretary-treasurer (reëlected).

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The Horse and Mule Association of America will convene for its 20th annual meeting at the Palmer House in Chicago, December 6.

Among the contributors and subjects scheduled for the program are H. W. Schoening and Benjamin Schwartz of the Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C., who will speak on "Equine Encephalomyelitis" and "Parasites in Horses and Mules," respectively.

Thos. P. Cooper, director of the Kentucky Agricultural Experiment Station, Lexington, Ky., will discuss periodic ophthalmia, and B. J. Errington of that station will present some information of direct interest to horse and mule owners.

War's demand for horses and mules—present and probable—will be reviewed by competent observers. This session promises to be one of the stellar features of the meeting.

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The program of the 43rd annual meeting of the United States Live Stock Sanitary Association, which will be held at the Morrison Hotel, Chicago, December 6-8, contains a long list of important subjects and prominent figures of the livestock sanitary service and the field of research appertaining thereto. For the first time in the long history of this quasi-

governmental society, a small animal problem is up for deliberation. Rabies will be discussed at length by capable authorities. Dean Rusk of the College of Agriculture, University of Illinois, will deliver the opening address.

Indiana

The third annual small animal clinic of the Northwestern Indiana Veterinary Association was held on November 29 at Glenn L. Ebright's hospital in Hammond.

Iowa

The 29th annual meeting of the Eastern Iowa Veterinary Association, held at the Hotel Montrose in Cedar Rapids, October 17-18, V. B. Vanderloo of Dubuque presiding, attracted a registered attendance of 219 veterinarians and 126 ladies.

Literary sessions on both days of the convention provided a comprehensive coverage of important topics and problems of the hour. The subject contributions and speakers were as follows:

Studies on the Virus-Type Equine Encephalomyelitis—H. E. Biester, Iowa State College, Ames.

Mouldy Corn Poisoning in Horses—L. H. Schwarte, Iowa State College.

The Physiology of Digestion—E. A. Hewitt, Iowa State College.

Bang's Disease in Cattle—J. A. Barger, U. S. bureau of animal industry, Des Moines.

Cattle Diseases—R. R. Dykstra, Kansas State College, Manhattan, Kan.

Poultry Practice—R. Fenstermacher, University of Minnesota, St. Paul, Minn.

Diseases of Farm Cats—Wayne H. Riser, Des Moines.

Introductory address to literary program of Wednesday afternoon, October 18—Hon. Mark G. Thornburg, Des Moines.

The A.V.M.A.: Your Association and Mine—Cassius Way, New York, N. Y.

Address on the present program of the A.V.M.A.—H. D. Bergman, Iowa State College.

Trichinosis in Swine—E. S. Dickey, Ottumwa.

Studies on Swine Influenza—Richard E. Shope, Rockefeller Institute for Medical Research, Princeton, N. J.

The Effect of High Temperatures on Hog-Cholera Virus and Anti-Hog-Cholera Serum—J. D. Ray, Omaha, Neb.

History and Demonstration of the Past and Present Technic of Serum and Virus Administration—Fred M. Maxfield, Gilman (assisted by John B. Bryant, Mount Vernon).

Lively discussions followed each presentation. Question boxes designed for the various topic sessions were an added feature of the program.

The spacious and artistically appointed Crystal room of the Hotel Montrose was the scene of the Association's banquet and dance, attended by 257 veterinarians and their ladies. Toastmaster Ashe Lockhart of Kansas City, Mo., introduced the following guests, each of whom gave a brief talk: W. B. Holmes of Springfield, Ill.; Earl R. Kennedy of Moline, Ill.; H. G. Dow of Fort Madison; Frank M. Wilson of Mechanicsville; and Lieutenant Governor B. B. Hickenlooper of Cedar Rapids.

The U. S. bureau of animal industry placed on display during the meeting an excellent exhibit of wax replicas of histological and pathological meat-inspection specimens and a collection of mounted animal parasites. This exhibit was arranged through the coöperation of John Morrell & Company, Ottumwa, and Wilson & Co., Cedar Rapids. W. A. Cornell of the South Omaha B.A.I. meat-inspection force was in charge of the display, and rightfully, too, for he is the artist who molded and painted the replicas.

Here are the men who will direct the Eastern Iowa association's activities during the coming year: Standing (left to right), A. R. Stephenson of Bennett; D. N. Voetberg of Traer; and C. A. Stewart of Tripoli, all trustees. Seated (left to right), S. G. Paul of Clarence, treasurer; C. L. Crider of Elkader, vice-president; I. W. Moranville of Durant, president; and H. E. Tyner of New London, secretary.

H. E. TYNER, *Secretary.*

Louisiana

With the eradication of the dreaded fever tick from Louisiana in recent years, the state's livestock industry has developed rapidly. Concomitantly, Louisiana veterinarians are finding a more fertile field for their activities, and the returns to owners from raising cattle have practically doubled during the past five years.

In addition to the growth of the beef-cattle industry, dairying has been developed considerably in many sections of the state.

Maryland

Approximately 150 veterinarians attended the annual meeting of the Maryland State Veterinary Medical Association, held at the

University of Maryland, College Park, on November 1-2, T. A. Ladson of Olney presiding.

A symposium on Bang's disease was conducted by A. E. Wight, chief of the tuberculosis eradication division of the federal bureau of animal industry; Melvin Rabstein, federal coöperative agent; and Mark Welsh, Maryland state veterinarian. Adolph Eichhorn, director of the Animal Disease Station at Beltsville, Md., discussed current veterinary problems. A. L. Brueckner and L. J. Poelma of the Maryland Live Stock Sanitary Service spoke on equine encephalomyelitis and bovine trichomoniasis, respectively. "Diseases of Foals" was presented by J. N. Frost of Cornell University, Ithaca, N. Y., and Lt. Col. L. C. Ewen and Capt. Williams of the Front Royal remount station, Front Royal, Va., discussed their important work on equine breeding diseases.

A clinic and several discussions on small animals were conducted by E. B. Dibbell of Baltimore; Otto Stader of Ardmore, Pa.; and John D. Gadd of Towson. H. M. DeVolt and C. R. Davis of the Maryland Live Stock Sanitary Service presented a poultry clinic and demonstration. The large animal clinic was capably staged by Drs. Frost and Gadd.

In the evening, a buffet dinner, minus speeches of any kind, was held for the members and guests at the College Park laboratories. Maryland oysters prepared in several ways and chicken a la Maryland were the *pièces de résistance*. Accordion and violin music during the evening aided those who like to harmonize and provided an obligato for the conversations of the others.

Officers elected for the coming year are: John D. Gadd, president; J. W. Spranklin of Baltimore, 1st vice-president; A. L. Brueckner of College Park, 2nd vice-president; and Mark Welsh of College Park, secretary-treasurer. The Association's board of directors consists of the following: J. P. Turner of Washington, D. C. (1940); John McClure of Hyattsville (1941); J. E. Gilfillan of Galena (1942); Walter Campbell of Bel Air (1943); and J. W. Hughes of Elkton (1944).

MARK WELSH, *Secretary.*

Massachusetts

During the early years of the Angell Memorial Animal Hospital in Boston, 20 to 30 horses were treated each day. During the entire year of 1938, only 115 horses were brought to that hospital. This famous animal refuge still hospitalizes horses, as 46 of these 115 head were kept under care for a few days to several weeks.—*From "Our Dumb Animals."*

Michigan

The following resolution was adopted by the Michigan State Veterinary Medical Association at their 57th annual meeting, June 28, 1939.

WHEREAS, The use of biological products which contain live pathogenic organisms by untrained persons is a well-known cause of serious losses to the livestock industry; and

WHEREAS, The State of Michigan attempts to prevent these losses by requiring all persons using, selling, or distributing any vaccine which contains the virus of any disease of live stock to report immediately such use, sale, or distribution to the Michigan Department of Agriculture and limits the use of hog-cholera virus to veterinarians; and

WHEREAS, Large amounts of such products are shipped into Michigan direct to farmers, garbage feeders, and other unqualified persons, to the certain detriment of the livestock industry of Michigan; and

WHEREAS, All concerns that manufacture and ship biological products interstate are licensed and supervised by the U. S. bureau of animal industry;

Therefore, The Michigan State Veterinary Medical Association in convention assembled does hereby respectfully petition the U. S. bureau of animal industry to coöperate with the Michigan livestock sanitary officials by doing everything within their power to see that all interstate shipments of such products are reported to the Michigan Department of Agriculture as required by law. The establishment of federal regulations to control the interstate shipment of live, disease-producing organisms is suggested.

Minnesota

The Tri-State Veterinary Medical Association held a meeting at the Hotel Winona, Winona, on November 9.

Frank Breed of Lincoln, Neb., presented a paper on virus diseases; C. L. Crider of Elkader, Iowa, spoke on bovine restraint; C. L. Campbell of St. Louis, Mo., presented a paper on swine erysipelas; and C. P. Fitch of University Farm, St. Paul, Minn., gave an account of the Memphis meeting of the A.V.M.A.

Dinner was served to 90 members and their guests in the Blue room of the hotel. Cards and a theatre party comprised the ladies' entertainment.

P. H. RIEDE, *Secretary.*

Missouri

Forty veterinarians attended the two-day poultry congress held at Columbia, November 7-8, by the veterinary department of the University of Missouri in coöperation with the Missouri Veterinary Medical Association.

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Kansas City made the headlines in *Certified Milk* for September, 1939, as the No. 1 city for its size in the sale of certified milk and

the discipline of the commission under which that class of milk is produced. The sales for 1938 were 1,494,622 quarts as compared with 234,266 but seven years ago (1932).

New Jersey

The Veterinary Medical Association of New Jersey is again coöperating in the program arrangements for New Jersey Agricultural Week, to be held in Trenton, January 23-26, 1940. Last year the Association sponsored several talks by prominent veterinarians on subjects of interest to the dairymen and horsemen of the state. The results were so gratifying that Secretary of Agriculture Allen requested the Association to help again in this project. Cassius Way of New York City, president of the national association, has been asked to speak on breeding and sterility problems in horses, and C. F. Clark of East Lansing, Mich., on breeding and sterility problems in dairy cattle.

New Mexico

At the seventh annual meeting of the New Mexico Veterinary Association, held at Clovis, October 27-28, E. E. Kraus of Clovis was elected president; R. M. Olbeter of Clayton, vice-president; and F. H. Sharp of Albuquerque, secretary-treasurer.

New York

Cornell University's 32nd annual conference for veterinarians will be held at Ithaca, January 10-12, 1940. The length of the conference has been increased from two to three days. The New York conference is the first one of its kind to be organized. The late Veranus A. Moore, onetime dean of the New York State Veterinary College, was its sponsor. In the years to follow other institutions founded short courses of the same general order.

Ohio

Last year the Ohio State Veterinary Medical Association voted to provide funds for awards to groups exhibiting live stock at the state fair. A $15.00 sweepstakes award in the 4-H club group and a similar award in the vocational agriculture group were approved by the Association's members. These prizes were announced in the premium list of the 1939 Ohio State Junior Fair for boys and girls.

It so happened that both winners were exhibitors of sheep. Richard S. Bame of Arlington won the 4-H club prize and Marvin Shinaberry of Fredericktown was the winner in the vocational agriculture sweepstakes.

Pennsylvania

The annual meeting of the Pennsylvania State Veterinary Medical Association was held at Harrisburg, October 24-25.

Papers were presented by V. C. Moyer of Upper Darby—Some Fundamentals of Dairy Inspection, W. J. Lentz of the University of Pennsylvania, Philadelphia—Nutrition and Diet, and A. H. Craige, Jr., of the University of Pennsylvania—Blood and Urine Analysis.

This meeting was the occasion of a dinner in honor of Louis A. Klein, professor of veterinary hygiene, University of Pennsylvania. The principal speaker was W. H. Ivens of Ardmore. Due to a sudden illness that overtook him just a few minutes before the dinner, Dr. Klein was unable to attend, much to the regret of every-

A. HENRY CRAIGE, JR.
A.V.M.A. Secretary for Pennsylvania.

Tennessee

According to A. C. Topmiller, there were 16 outbreaks of erysipelas in 16 counties of the state during the month of October; 4 outbreaks of blackleg in 3 counties and 4 of hemorrhagic septicemia in 3 counties. Forty outbreaks of hog cholera were reported during the month from 16 counties, Sumner county, with 16 outbreaks, being by far the most seriously affected.

Texas

The Houston Veterinary Association, coöperating with the central office of the A.V.M.A., presented a framed picture to J. Gilbert Horning, corresponding secretary of the Houston group, showing him in a characteristic demonstration of his work on endoscopy. The picture was taken by the official A.V.M.A. photographer at the Memphis small animal clinic and appeared in the October 1939 issue, p. 409.

Wyoming

Officers elected by the Wyoming Veterinary Medical Association at a meeting held at the Gladstone Hotel in Casper, October 30-31, are G. H. Good of Wheatland, president; A. B. Kight of Casper, vice-president; and H. D. Port of Cheyenne, secretary-treasurer.

COMING MEETINGS

Conference of Official Research Workers in Animal Diseases of North America. Hotel Sherman, Chicago, Ill. December 5, 1939. (Research workers employed by the U. S. government, by any national, state or provincial government in North America, or by any endowed institutions and engaged in the investigation of animal diseases are eligible for membership in the Conference.) William H. Feldman, secretary, The Mayo Foundation, University of Minnesota, Rochester, Minn.

Small Animal Hospital Association. Los Angeles, Calif. December 5, 1939. R. W. Gerry, secretary, 8474 Melrose Ave., Los Angeles, Calif.

Horse and Mule Association of America. Palmer House, Chicago, Ill. December 6, 1939. Wayne Dinsmore, secretary, 407 S. Dearborn St., Chicago, Ill.

New York City, Veterinary Medical Association of. Hotel New Yorker, New York, N. Y. December 6, 1939. J. J. Merenda, secretary, 136 W. 53rd St., New York, N. Y.

National Association of Bureau of Animal Industry Veterinarians. Morrison Hotel, Chicago, Ill. December 6-8, 1939. F. A. Imler, secretary, P. O. Box 187, Kansas City, Kan.

United States Live Stock Sanitary Association. Morrison Hotel, Chicago, Ill. December 6-8, 1939. L. A. Merillat, secretary, 221 N. La Salle St., Chicago, Ill.

Dallas-Fort Worth Veterinary Medical Society. Dallas, Texas. December 7, 1939. H. V. Cardona, secretary, 2736 Purington Ave., Fort Worth, Texas.

Houston Veterinary Association. Houston, Texas. December 7, 1939. W. T. Hufnall, secretary, 1612-14 E. Alabama Ave., Houston, Texas.

Chicago Veterinary Medical Association. Hotel Sherman, Chicago, Ill. December 12, 1939. W. A. Young, secretary, 157 W. Grand Ave., Chicago, Ill.

Nebraska State Veterinary Medical Association. Omaha, Neb. December 12-13, 1939. J. D. Sprague, secretary, David City, Neb.

Southeastern Michigan Veterinary Medical Association. Medical Arts Bldg., 3919 John R St., Detroit, Mich. December 13, 1939. F. D. Egan, secretary, 17422 Woodward Ave., Detroit, Mich.

St. Louis District Veterinary Medical Association. Melbourne Hotel, St. Louis, Mo. December 13, 1939. J. P. Torrey, secretary, 610 Veronica Ave., East St. Louis, Ill.

Long Island Veterinary Medical Association. Long Island, N. Y. December 14, 1939. Herman Tax, secretary, State Institute, Farmingdale, Long Island, N. Y.

Western New York Veterinary Medical Association. Buffalo, N. Y. December 14, 1939. F. F. Fehr, secretary, 243 S. Elmwood Ave., Buffalo, N. Y.

Kansas City Veterinary Medical Association. Kansas City, Mo. December 18, 1939. S. J. Schilling, secretary, Box 167, Kansas City, Mo.

San Diego County Veterinary Medical Association. Zoölogical Research Bldg., Balboa Park, San Diego, Calif. December 18, 1939. Paul D. DeLay, secretary, State Poultry Pathological Laboratory, Balboa Park, San Diego, Calif.

Southern California Veterinary Medical Association. Chamber of Commerce Bldg., Los Angeles, Calif. December 20, 1939. Charles Eastman, secretary, 725 S. Vancouver Ave., Los Angeles, Calif.

Keystone Veterinary Medical Association. School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa. December 27, 1939. C. S. Rockwell, secretary, 4927 Osage Ave., Philadelphia, Pa.

Massachusetts Veterinary Association. Hotel Westminster, Copley Square, Boston, Mass. December 27, 1939. H. W. Jakeman, secretary, 44 Bromfield St., Boston, Mass.

California Veterinary Conference (Division of Veterinary Science, University of California, and California State Veterinary Medical Association coöperating). College of Agriculture, University of California, Davis, Calif. January 2-5, 1940. Charles J. Parshall, secretary, 319 B St., Petaluma, Calif.

Ohio State Veterinary Medical Association. Deshler-Wallick Hotel, Columbus, Ohio. January 3-5, 1940. R. E. Rebrassier, secretary, Ohio State University, Columbus, Ohio.

Minnesota State Veterinary Medical Society. Hotel Radisson, Minneapolis, Minn. January 8-9, 1940. H. C. H. Kernkamp, secretary, University of Minnesota, St. Paul, Minn.

Intermountain Livestock Sanitary Association. Salt Lake City, Utah. January 8-10, 1940. D. E. Madsen, secretary, Utah Experiment Station, Logan, Utah.

Mississippi State Veterinary Medical Association. Alacza Hotel, Clarksdale, Miss. January 15-16, 1940. E. H. Durr, secretary, Clinton Blvd., Jackson, Miss.

New Jersey, Veterinary Medical Association of. Hotel Douglas, Newark, N. J. January 16-17, 1940. J. G. Hardenbergh, secretary, c/o Walker-Gordon Laboratory Co., Plainsboro, N. J.

Post-Graduate Short Course for Veterinarians. Veterinary Hospital, Michigan State College, East Lansing, Mich. January 22-26, 1940. Ward Giltner, dean, Division of Veterinary Science, Michigan State College, East Lansing, Mich.

State Board Examination

Oklahoma Board of Veterinary Medical Examiners. State Capitol Bldg., Oklahoma City, Okla. January 10-11, 1940. W. C. McConnell, secretary, Holdenville, Okla.

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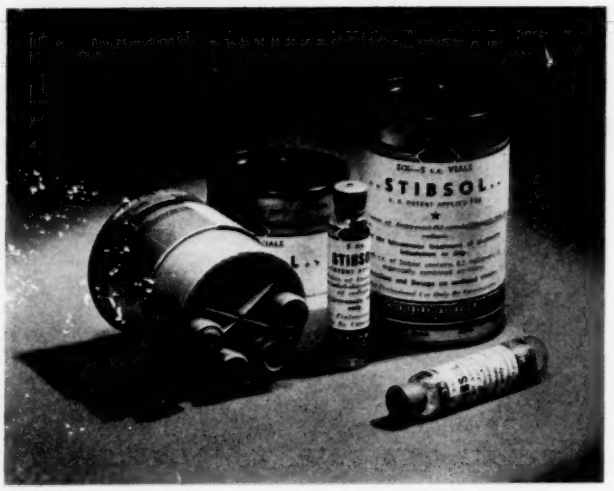

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